ENDOCRINOLOGY

THE BULLETIN OF
THE ASSOCIATION FOR THE STUDY
OF INTERNAL SECRETIONS

VOLUME 25 DECEMBER, 1939 NUMBER 6

AN ANALYSIS OF 583 GLUCOSE TOLERANCE TESTS

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SINCE 1931 every case of glycosuria found on routine examination of students at the University of Minnesota Students' Health Service has been further studied by means of a glucose tolerance test. This report covers an analysis of 583 such cases studied from 1931 to 1937, inclusive.

Determination of presence of glycosuria. A positive reaction to the standard Benedict qualitative test was taken as evidence of glycosuria. No known diabetics were included in this study.

History and physical examinations. All individuals presenting themselves for glucose tolerance tests because of glycosuria were questioned regarding the following.

(*a*) Presence or absence of family history of diabetes.
(*b*) Frequency of urination, e.g., polyuria and nocturia.
(*c*) Increased thirst and appetite or rapid gain or loss of weight.
(*d*) History of fatigue, acute or chronic infections, boils, carbuncles and previous glycosuria.
(*e*) The type of diet the patient was eating previous to finding of glycosuria.

A complete physical examination was made at the time the glycosuria was found. Particular attention was given as to whether the patient was normal, under or overweight.[1] If there was evidence of infection the tolerance test was postponed until it had subsided. If an abnormal type of diet was eaten (ulcer, allergical, etc.) the test was delayed two or three weeks during which time a normal diet was prescribed.

Technic of the glucose tolerance test. The patient was instructed to fast from 6:00 P.M. the evening preceding the test until he presented himself at the laboratory at 8:00 A.M. Fasting blood and urine samples were obtained; then 100 gm. of glucose dissolved in 250 cc. of water were ingested and blood and urine samples were obtained at intervals of ½, 1, 2, and 2½ hours after the drink.

Blood sugar determinations were made by the Gibson micro method (1) and the urine was tested for glucose with Benedict's qualitative solution.

[1] Normal weight, 90 to 110% variation from the ideal weight as calculated from standard height-weight tables.
Underweight, less than 90% of ideal weight.
Overweight, 110% or more of ideal weight.

DATA

Age of subjects. The average age of the subjects studied was 22.7 years (range, 14 to 61 years).

Incidence of glycosuria. Among 51,096 routine urine examinations glycosuria occurred in 583 or 1.14% of the cases.

In 33,125 urine examinations among males glycosuria occurred in 368 or approximately 1.11% while in 17,971 such examinations among females glycosuria was found in 215 or 1.19%.

Incidence of glycosuria during tolerance test and its effect on mean blood sugars. In the series of 583 tolerance tests there were 368 males and 215 females. One hundred and eighty-six, or 48.2% of the males, and only 57, or 26.5% of the females, had glycosuria on one or more occasions during the

TABLE 1

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
186 tolerance tests on males with glycosuria during test	M.[1]	105	173	163	125	112
	S.D.[2]	38	51	72	78	79
	R.[3]	62–418	73–452	65–494	46–576	29–515
182 tolerance tests on males with no glycosuria during test	M.	99	147	125	102	94
	S.D.	15	29	31	25	24
	R.	61–161	75–253	64–215	52–177	50–189
57 tolerance tests on females with glycosuria during test.	M.	115	174	166	152	139
	S.D.	55	68	82	52	106
	R.	65–411	95–513	63–514	65–533	50–512
158 tolerance tests on females with no glycosuria during test	M.	97	138	115	103	97
	S.D.	15	26	29	24	21
	R.	59–169	56–237	59–215	54–193	43–161

[1] M., Mean. This figure in all tables represents mean blood sugar value expressed as mg. per 100 cc. of blood.
[2] S.D., Standard Deviation.
[3] R., Range.

test. Why almost double the number of males showed glycosuria is difficult to explain, as all subjects were given 100 gm. of glucose and the test was carried out under the same conditions in both sexes.

One would naturally expect to find slightly higher values in the tolerance tests on individuals having glycosuria during the test than those with no glycosuria. Table 1 compares the mean values of the two groups.

Incidence of disturbed carbohydrate metabolism. Ninety, or 15.4% of 583 individuals studied had evidence of a definitely disturbed glucose tolerance. Table 2 compares the mean blood sugar values of the entire group of 583, the disturbed group of 90, and a normal group of 83 cases (2), using the same technic of blood sugar determination. The mean blood sugar values of the 493 of 583 cases that were judged as normal also are shown. The average age of the disturbed group was 24.29 years (range 17 to 61) compared with an average age for the entire group of 22.7 years (range 14 to 61).

Sex and its relation to tolerance tests. That there were no essential dif-

TABLE 2

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
583 routine glucose tolerance tests	M.	102	155	139	115	105
	S.D.	30	44	58	60	60
	R.	57–418	56–513	59–514	46–576	29–515
90 disturbed glucose tolerance tests	M.	134	216	220	192	180
	S.D.	61	67	89	117	118
	R.	64–418	122–513	114–514	66–576	66–515
83 normal glucose tolerance tests	M.	92	135	115	92	84
	S.D.	12	25	29	20	18
	R.	53–131	79–208	67–212	41–155	33–121
Remaining 493 glucose tolerance tests after 90 disturbed cases were removed	M.	96	145	124	101	91
	S.D.	14	37	31	22	22
	R.	57–175	56–413	59–418	46–171	29–158

ferences between the response of the males and females to the glucose tolerance test is indicated by table 3.

Relation of history of previous glycosuria to mean blood sugar values. One would expect higher blood sugar values during the tolerance test among individuals who gave a history of previous glycosuria. Only 49 of 583 sub-

TABLE 3

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
386 routine tolerance tests on males	M.	102	159	144	114	103
	S.D.	29	44	58	59	60
	R.	61–418	73–454	64–495	46–576	29–515
215 routine tolerance tests on females	M.	101	148	129	116	108
	S.D.	32	45	54	61	60
	R.	57–416	56–513	59–514	54–533	43–513

jects gave such a history, and 33 or 67.3% of these 49 subjects had glycosuria sometime during the tolerance test. Table 4 shows the mean blood sugar values of this group. It should be noted that all blood sugar values are slightly elevated except the fasting specimen. Some of these individuals might have had a mildly disturbed carbohydrate metabolism when glycosuria was first found, but because of the fact that another urine specimen may have shown no sugar they were not treated at that time. Thus, during the elapsed time between the first and second finding of glycosuria one might expect a further loss of carbohydrate tolerance. It is important to note that, had only a

TABLE 4

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
49 tolerance tests on subjects with history of glycosuria	M.	113	166	164	147	143
	S.D.	35	60	83	102	109
	R.	65–256	60–418	60–415	40–570	40–519

fasting blood sugar been obtained instead of a glucose tolerance test, a disturbance of tolerance would not have been noted at this time in this group.

Relation of weight to the tolerance test. Joslin (3) and others have frequently called attention to the rôle of obesity in the etiology of diabetes. Subjects that were overweight and had glycosuria might be expected to show higher blood sugar values during the tolerance test than normal or underweight subjects in a routine study. Also, if one selected obese patients (20% or more overweight) having glycosuria, considerably higher blood sugar values might be expected. Table 5, however, shows none of the fore-

TABLE 5

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
Tolerance tests on 63 overweight males	M.	101	158	153	121	102
	S.D.	21	34	47	48	51
	R.	63–195	96–268	65–336	64–379	40–333
Tolerance tests on 54 underweight males	M.	104	167	151	117	109
	S.D.	22	38	63	63	66
	R.	67–208	110–282	72–474	46–437	29–416
Tolerance tests on 246 normal weight males	M.	102	159	141	111	101
	S.D.	35	47	60	61	60
	R.	61–418	73–454	64–498	52–576	39–515
Tolerance tests on 30 overweight females	M.	96	145	134	119	107
	S.D.	15	31	40	40	30
	R.	64–133	91–235	75–241	69–250	50–276
Tolerance tests on 39 underweight females	M.	107	161	134	120	111
	S.D.	54	71	86	89	87
	R.	57–419	82–513	59–514	54–514	50–517
Tolerance tests on 144 normal weight females	M.	101	144	127	114	107
	S.D.	26	36	45	55	56
	R.	61–316	56–377	62–453	59–536	43–480
Tolerance tests on 37 subjects 20% or more overweight	M.	97	149	142	117	102
	S.D.	16	31	38	34	31
	R.	63–134	96–256	65–209	60–204	59–176

going assumptions to be true; in fact, in both sexes the underweight and normal weight groups had higher blood sugar values than the overweight group, and even in the obese group the blood sugar values were practically the same or below those of the underweight group.

Dividing the entire group of 583 according to weight, one finds that 67.7% were normal weight, 16.1% were underweight and 16.1% were overweight. That this is a normal weight distribution among the general college population is borne out by the fact that among 4652 students with approximately the same sex distribution that is present in this study of 583 cases there were 66.4% normal weight; 17.1% overweight and 16.5% underweight. That the group which had disturbed tolerance tests had essentially the same distribution of normal, under-, and overweight individuals as the whole group is demonstrated by the fact that 58.6% were normal

weight, 21.8% were underweight and 19.5% were overweight. Table 6 shows the mean blood sugar values of each of the latter group. Note that the overweight group has the lowest values.

TABLE 6

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
51 disturbed tolerance tests on normal weight subjects	M.	135	216	217	195	189
	S.D.	63	68	96	122	122
	R.	64–418	122–454	114–497	66–576	66–512
19 disturbed tolerance tests on underweight subjects	M.	143	235	247	198	187
	S.D.	71	80	100	138	136
	R.	96–404	59–513	56–514	84–531	79–515
17 disturbed tolerance tests on overweight subjects	M.	118	194	199	178	158
	S.D.	30	35	52	64	77
	R.	73–195	138–262	144–337	108–376	79–338

There were in this group of 583 subjects 16 individuals (8 males and 8 females) having glycosuria, a family history of diabetes and obesity. Their average age was 21.69 years (range 17 to 36 years). This group was selected for special study because it was felt that if any single group should show a predisposition to diabetes as indicated by the glucose tolerance test this group should. Table 7 shows the mean values to be entirely within normal limits.

TABLE 7

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
16 tolerance tests on subjects with glycosuria, obesity and family history of diabetes	M.	91	148	149	116	107
	S.D.	13	26	37	45	46
	R.	73–119	122–217	97–241	60–253	51–266

From all these data presented it is impossible to demonstrate that obesity per se plays any part in the causation of a disturbed tolerance test. In fact, one would predict on a basis of our findings that individuals in the underweight group would be candidates for disturbed carbohydrate metabolism much more frequently than those in the overweight group. Thus one may conclude that in this age group obesity plays no important rôle in the etiology of a disturbed carbohydrate metabolism.

TABLE 8

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
583 tolerance tests on entire group of subjects	M.	102	155	139	115	105
	S.D.	30	44	58	60	60
	R.	57–418	56–513	59–514	46–576	29–515
109 tolerance tests on subjects with family history of diabetes	M.	101	155	141	113	108
	S.D.	36	45	62	57	60
	R.	61–418	56–453	62–489	46–512	29–479

Relation of family history of diabetes to the tolerance test. One hundred and nine of the 583 subjects having glycosuria gave a family history of diabetes. Table 8 shows their mean blood sugar values compared with those of the entire group.

Among 109 individuals with a family history of diabetes the incidence of disturbed tolerance tests was 17.4% compared to the incidence of 14.9% of disturbed tolerance tests among the remaining 474 individuals with no family history of diabetes.

Thus from these data one may conclude that heredity cannot be considered an important etiological factor in the production of a disturbed carbohydrate metabolism in this age group. The author realizes years must pass before the hereditary character of diabetes can be definitely disproved or proved in accordance with the theories of Joslin (3), Pincus and White (4) and others.

TABLE 9

		Fasting	½ hr.	1 hr.	2 hr.	2½ hr.
532 tolerance tests on subjects under 30 years of age	M.	102	155	138	114	104
	S.D.	31	44	57	60	60
	R.	57–418	56–513	59–514	46–576	29–515
36 tolerance tests on subjects 30 to 39 years of age	M.	97	151	137	110	96
	S.D.	11	36	43	32	34
	R.	74–127	82–254	64–236	63–214	50–238
15 tolerance tests on subjects 40 years of age or older	M.	119	169	184	165	152
	S.D.	46	52	77	88	85
	R.	62–239	101–290	102–378	88–375	64–334

Age and its effect on the tolerance test. Malmros (5) and other workers have called attention to the deleterious effect of age on the response of an individual to the glucose tolerance test. Five hundred and thirty-two, or approximately 91.2% of the cases in this study were under 30 years of age; 36, or 6.2% were from 30 to 39 years of age and 15, or approximately 2.6% were over 40 years of age. Table 9 compares the tolerance tests of each group according to age. It shows no apparent effect on tolerance until after the age of 40, and it should be noted that there are only 15 cases in this age group, making it difficult to draw any definite conclusions as to whether or not age adversely affects the glucose tolerance test.

DISCUSSION

The general incidence of glycosuria in this age group (mean 22.7 years) is about 1%. Individuals having glycosuria on one occasion have about 1 chance in 6 of having a disturbed glucose tolerance, indicating a diminished carbohydrate metabolism of some degree. It is in this group that an attempt has been made to learn more about the development of true diabetes and its prevention by various methods of treatment. A report of this work which has been carried on at the University of Minnesota Students' Health Service during the last 7 years will soon be published.

Our data do not support the view that obesity is an important factor in the etiology of a disturbed carbohydrate metabolism; in fact, the underweight individuals were found to have slightly higher mean blood sugar values than the normal or overweight groups. Even the obese (20% or more overweight) group had mean blood sugar values that approached normal figures. These facts, together with the finding that there were only 17 overweight individuals in the total of 90 subjects who had disturbed glucose tolerance tests, seem to indicate that at least in this age group obesity is not a predisposing factor to a disturbed carbohydrate metabolism.

Family history of diabetes, that is heredity, does not appear to be a major influence in the causation of a disturbed carbohydrate metabolism in this age group. There were only 19, or 17.4% of individuals with a family history of diabetes who had evidence of a disturbed carbohydrate metabolism. In other words, 82.6% of those individuals with a family history of diabetes had perfectly normal glucose tolerance tests, even though they had shown glycosuria in a routine examination. The final answer to this question, however, will not be forthcoming until several generations have come and gone. In this regard an attempt will be made to follow this group of individuals over a period of years to find if a larger proportion of them develop diabetes than of the group with no family history of diabetes.

Individual variation in the glucose tolerance test in this large group was interesting. For instance, fasting blood sugars of 60 to 65 were commonly observed, and the post-ingestion blood sugars sometimes did not rise above 115 to 120. Several subjects at the end of the test with blood sugars of 40 to 60 had definite evidence of hypoglycemic reactions, with perspiration and involuntary muscle contractions. Others with the same blood sugar values at the end of the test had no such symptoms. Thus, one sees individual variations in reaction to the same blood sugar level which may account in part for the fact that some diabetics have insulin reactions more frequently in the presence of blood sugar values near the lower limits of normal.

Finally, it should be stated that this study was started and is being carried on in an attempt to evaluate the clinical significance of glycosuria and demonstrate the value of the early diagnosis of a disturbed carbohydrate metabolism and the prevention of the development of clinical diabetes mellitus.

SUMMARY

A study of 583 routine glucose tolerance tests on patients with glycosuria is presented. The incidence of glycosuria in routine urine examinations was 1.14%. No sex difference was noted in the incidence of glycosuria or mean blood sugar values during the tolerance test.

About 15.5% of patients with glycosuria on routine examination will have evidence of a definitely disturbed carbohydrate metabolism as indicated by the glucose tolerance test. In this age group the mean blood sugar values during the tolerance test of the overweight group were lower than those of the normal weight group, and the normal weight group were lower than those

of the underweight group. Thus, obesity does not appear to predispose to a disturbance of carbohydrate metabolism in this age group.

The question of the importance of family history as a predisposing factor to the development of a disturbed tolerance and ultimate diabetes is raised, as subjects with a family history of diabetes had no higher mean blood sugar values during the tolerance test than did those with no such history. Only 17 of the 109 subjects with a family history of diabetes had disturbed tolerance tests. It is noted that there were wide variations in individual responses to the glucose tolerance even in the so-called normal group.

The suggestion is made that routine tolerance tests on patients having glycosuria may be an aid to early diagnosis and treatment of a disturbed carbohydrate metabolism, thus preventing the onset of clinical diabetes (6).

Grateful acknowledgment is hereby given to Misses Ryan, Benson, Williams, Erskine, Hoiland, Gillig, Ringbloom and Zschiesche; who, over the period of years of this study have aided me in the collection of specimens; and to Miss Martha Lavell, who has done the statistical analysis of our data.

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THE RESPONSE OF CHRONICALLY FATIGUED NEUROTIC PATIENTS TO ADRENAL CORTEX THERAPY[1]

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FAILURE IN FUNCTION of the adrenal glands has long been recognized in Addison's disease, although the hormone elaborated by the medullary portion of these glands was earlier considered of the greater importance. The internal secretion of the adrenal cortex, investigated later, has been found to be the one that possesses life-sustaining qualities for Addisonian patients. What it can accomplish for persons who do not suffer from an irreversible degeneration of the adrenal cortical tissue, but whose symptoms do suggest a hypofunctioning (whether reversible or irreversible) of this endocrine gland, has been the subject of more recent studies.

Deficiency symptoms. In cases of Addison's disease in man (deficiency or loss of cortico-adrenal tissue being typical of this disorder), Hartman and coworkers, Britton, Loeb, Zwemer, Hoskins and many other investigators have described the following typical deficiency symptoms: gastrointestinal disturbances, loss of appetite and weight; lowered blood pressure, low heat production, fall of the basal metabolic rate (to −20 or −25), disturbance in the metabolism of carbohydrate, fall of sodium content of the plasma and rise of potassium content, rise of blood urea, reduction in resistance to infection, liability to intoxication by substances ordinarily nontoxic; pigmentation; gonadal malfunctioning, as cessation of menstruation and loss of libido; asthenia, fatigability, irritability, muscular twitching, insomnia, apathy, failure of memory and judgment, restlessness and anxiety tending to progress to dementia, coma and convulsions in the final stage.

Excepting the bronzed pigmentation, which is paralleled by other changes in the skin, practically the entire symptom-complex just outlined is duplicated in adrenalectomized animals. Such differences as occur are ascribable to a sudden as opposed to a gradual deprivation, complicated as the latter typically is in man by tuberculosis.

Deficiency symptoms referable to the adrenal cortex may be confused with forms of hypopituitarism (1). Put in another way, part of the syndrome of chronic adrenal insufficiency may be due to a resulting diminution of

[1] Acknowledgement is made to the late Dr. Frederick Tilney, Director of the Neurological Institute, for permission to use its clinical material and for sponsoring this study.
We wish to express our thanks to Schieffelin & Co. for generous supplies of the glycortal extract used in this study.

pituitary activity (2). This pituitary involvement might be connected with the gonadal hypofunctioning already mentioned. A gonadotropic factor in the adrenal-cortex secretion may be postulated (3), to account for the precocious sex development accompanying certain cortical tumors. Whether or not via the pituitary, which showed hypertrophy, Corey and Britton (4) induced precocious sex-gland maturation in young rats by injections of cortical extract. Britton (5) suspects that the adrenal cortex secretes two hormones, one sex-stimulating and the other life-maintaining. Marrian (6) adduces considerable evidence for the presence of progesterone in the adrenal cortex.

Rogoff (7) reviews the possibilities of a functional interrelation between the adrenals and the parathyroids.

Considerable discussion has centered upon the relation between the adrenals and the metabolism of sodium and potassium. Loeb and Atchley (8) maintain that a regulatory effect on sodium metabolism is one of the functions of the adrenal cortex, and remark that sodium withdrawal without other procedure will bring about symptoms of acute adrenal insufficiency in either the Addisonian patient or the adrenalectomized dog; further, that such a dog will live for months without cortical extract if ingesting sufficient sodium. However, Loeb (9) had pointed out that the adynamia, hypotension, pigmentation, gastro-intestinal symptoms, neurological disturbances and hypoglycemia of Addison's disease might occur without a decrease in sodium. Stahl, Atchley and Loeb (10) found that strength increased strikingly in the adrenalectomized dog, following the administration of cortical extract, and before obvious changes occurred in the blood urea, sodium concentration or water content of the blood serum.

Kendall and Ingle (11) claim that a normal condition can be maintained in adrenalectomized animals only by giving cortin (a cortico-adrenal extract), or enormous amounts of sodium chloride with sodium citrate or sodium bicarbonate and with a minimal intake of potassium. These authors record their agreement with Selye, that the changes observed after adrenalectomy are symptoms, rather than the primary cause, of the condition of insufficiency.

Although endocrinologists seem to agree that an adrenal insufficiency less severe than that pertaining to Addison's disease has never been demonstrated as a clinical entity, it is not unreasonable to expect such *formes frustes*. The fatigability, weakness and depression of certain psychoneuroses may possibly be associated with adrenal hypofunctioning. Hypotension and poor circulation are common to many psychoneuroses and to Addison's disease. Attempts to make a therapeutic test for hypoadrenalism have been reported by Hartman, Greene, Bowen and Thorn (12) in cases of asthenia (see below). In adrenalectomized rats, it was found (13) that fatigability involved the reflex, the myoneural junction and the muscle, in a spinal reflex preparation.

Replacement therapy: injections; source and preparation. Active material was extracted from adrenal cortex by Rogoff and Stewart (14), through the use of various lipoid solvents and with the final product in aqueous solu-

tion. They were able to demonstrate this extract's potency on adrenalectomized animals.

Hartman (15, and elsewhere) and Hartman and Brownell (16) have since described methods for producing a potent, nontoxic extract, as have Swingle and Pfiffner (17), and Zwemer, Agate and Schroeder (18).

Rogoff (19) has commented: (*a*) that some of the cortical adrenal extracts may not have been sufficiently pure to warrant the conclusions drawn from their experimental use; (*b*) that certain samples were inert, even toxic; (*c*) that one must not disregard the stimulating effect of simple saline solutions given intravenously to adrenalectomized animals.

Results of injections. When adrenalectomized rats were given injections of adrenal cortex extracts, their fatigue time was lengthened (20), and more work was done (21). Adrenalectomized animals under cortin injection maintenance (12, 13) required larger amounts if they were exercised, exposed to cold or high temperatures, physically injured, or affected by toxins.

To human patients suffering from the obvious adrenal insufficiency of Addison's disease, Hartman and coworkers (12, and elsewhere) gave a series of subcutaneous injections of cortin (1 cc. of this extract contained the product of 40 gm. of cortex). Under this therapy their patients exhibited gains in weight, less insomnia, greater capacity for work as measured by the ergometer, sharper mental alertness, restoration of menses in amenorrhoeics and and increase in diminished libido (22). The amount of cortin required for maintenance of improvement was increased by intercurrent infections.

The patient's response to a salt-free diet has been suggested as a diagnostic test in suspected Addison's disase. This procedure is not in common use nor well standardized, and there seems to be no other certain diagnostic criterion of the disease being present.

Several 'replacement therapy' experiments have been carried out on cases of supposed adrenal insufficiency in a mild, partial or incipient form. Schaefer and Strickroot's (23) patients showed fatigability and low blood pressure, without psychoneurosis. They exhibited marked improvement after injections of adrenal cortex extract, whereas a parallel series of cases of post-influenzal prostration were not benefited by the same treatment. Hartman, Beck and Thorn (24) gave cortin injections to patients showing asthenia and fatigability, usually accompanied by anxiety tension. Fatigability was temporarily reduced in 10 of 16 cases, 6 of which were tested ergometrically. Irritability and anxiety were relieved, appetite and sleep improved and a sense of well-being was promoted.

Rowntree, Greene, Swingle and Pfiffner (25), in a series of neurasthenics and other psychoneurotics, did not detect improvement in the asthenia after injections of a cortical extract.

Liddell, Anderson, Kotyuka and Hartman (26) report marked relief in all the symptoms of experimental neurosis in sheep, through cortin injections. In this neurosis, produced by the conditioning method, the animals were highly uncooperative and excited and showed twitchings of the conditioned extremity.

Studies of normals, both animal and human, have demonstrated certain effects of extracts of adrenal cortex, so that some positive response to them demonstrates pharmacological action rather than certain evidence for cortical deficiency. For instance, in the treadmill-running normal dogs of Eagle, Britton and Kline (27), injections of cortical extract practically doubled the energy output ("energy calculated in 1000 kilogram-meters in excess of basal metabolism"). Some effect was still perceptible as long as 10 to 15 days after the last injection. The B.M.R. is not raised by cortin in normal animals (28). In normal sheep, the conditioned response has been found (26) increased by cortin, an effect which lasted over 24 days after the final injection.

Missiuro, Dill and Edwards (29), using 4 resting normal men, injected ½ to 1 cc. of Hartman's cortical extract daily for 3 to 5 days. Easy walking was performed more efficiently for some days after the injection period. For moderate or severe work, the blood pressure in the early stage of recovery was lower, and returned to its resting value sooner, than without cortin. No significant changes in the B.M.R. were recorded. Hartman (28) cites a similar finding by other workers of no change in the B.M.R. of normal human subjects under cortin influence.

Hartman, Greene, Bowen and Thorn (12) report results of cortin injections in 12 normal subjects. With all of these, of either sex and of ages ranging from 26 to 48 years, 1 cc. produced a perceptible effect, while 3 to 5 cc. brought about unquestioned effects lasting several hours. (Control injections of saline or of brain tissue extract were without effect.) Within an hour after a cortin injection, a subject generally fell asleep, awakening after another hour or so in a physically fitter condition, more active and alert. Appetite and sleep tended to improve in these experimental subjects and some of them gained from 50 to 500% improvement in performance on the ergometer within a few days after a course of cortin injections was begun. In 4 of the 6 women subjects, 3 to 5 cc. of cortin daily for 4 or 5 days brought on menstruation 3 to 5 days sooner than usual. The B.M.R.'s of normal human subjects were not affected.

Feeding results. As early as 1895, Oliver and Schaefer (30) obtained good results with glycerin extract of the adrenal gland given by mouth. During the last decade, a number of investigators have reported on the effective feeding of similar glycerin extracts of the cortex. Rogoff and Stewart (31) obtained as much effect through the oral administration of a cortical extract ('interrenalin') in the treatment of Addison's disease as others had claimed for extracts employed parenterally. Gordon, Severinghaus and Stark (32) report the oral use of several preparations of adrenal cortex, including 'glycortal' (Schieffelin & Co.): their cases of long standing adrenal insufficiency were promptly and definitely improved, while a few cases of asthenia from a series of psychasthenics and climacteric psychoneurotics showed improvement. Hoskins, Freeman and Linder (33, 34, 35), Bram (36) and others have reported specifically on the use of glycortal.

Bram gave this cortical extract, in doses of 9 to 12 ten-grain pills daily, to a dozen patients suffering from exophthalmic goiter. He reported improved

weight, reduced heart rate, lowered B.M.R., reduction of increased blood pressure to normal, lessened nervousness and fatigability. The average duration of adrenal cortex administration was 10 weeks, and relief from the most prominent toxic symptoms occurred 8 weeks sooner than with a control group of patients not receiving the glycerinated adrenal cortex.

Noting that schizophrenia generally was characterized by hypometabolism, at least suggestive of underfunctioning adrenals, Hoskins and Freeman (33) administered the glycortal preparation of cortex to a series of 10 adult male schizophrenics. The treatment was continued for a 10-week period. Dosage began with the equivalent of 100 grains (6.5 gm.) of fresh cortex daily, and was gradually raised to that of 450 grains (29.2 gm.) daily. In this experiment blood pressures were uniformly raised, from an initial average of 105.7 to a final of 132.5 mm. Hg, systolic, and from 69 to 84 mm. Hg, diastolic. After treatment was discontinued, blood pressures returned approximately to their initial levels. The patient's average weight showed an increase from 62.6 kg. to 65.4 kg. during the treatment. The B.M.R.'s showed a diphasic type of response to cortical medication. Early in the study period the average B.M.R. dropped from an initial of 87 to one of 84% of normal; after 6 weeks, the average stood at 92%; but at the end of 10 weeks it had fallen back to the initial 87.

Freeman, Linder and Hoskins (34) studied a second series of 9 schizophrenic patients more exhaustively, over 3 successive medication periods of 13, 9 and 5 weeks. The maximum dosage (of the glycortal preparation of cortical extract, as before) was a fresh-gland equivalent of 450 grains (29.2 gm.) daily. The average systolic blood pressure increased 34, 24 and 22 mm. Hg during the 3 periods, and the average diastolic, 20, 11 and 20. (Blood pressures were lower in the basal metabolism laboratory than on the wards.) The cardiovascular reactivity of the patients to change of posture and to exercise increased through the periods, and also through the hours of each day. The pulse rate was lowered, the body weight slightly raised. There was a slight rise in the B.M.R. under medication; statistical analysis proved this to be nonsignificant. The psychoses were not perceptibly ameliorated.

Using the same extract, in a daily dosage of the equivalent of 450 grains (29.2 gm.) of fresh cortical substance, Freeman and Hoskins (35) treated two series of normal control subjects. A pressor effect on the blood pressure was observed in but 18% of the controls, as against 79% of the schizophrenics. This effect on the normals was much smaller when it appeared at all.

The purpose of this study. The present study of the response of chronically fatigued psychoneurotic patients to adrenal cortex therapy was undertaken because of the striking similarity of the symptoms in such patients to those in the early stages of Addison's disease or adrenal cortex insufficiency. The success of Hartman and other workers (12, 22, 24 and 37) with cortin in treating neurotic patients indicated that further studies were in order. In spite of the fact that extracts of adrenal cortex injected subcutaneously are more potent than similar extracts given orally, the glycortal preparation was used because of ease of administration. The results cited above indicated

that this preparation was effective in patients with hypometabolism, low blood pressure, nervousness and fatigability.

Selection of patients. Cases were selected for the most part from the outpatient department of the New York Neurological Institute, in the Vanderbilt Clinic. Several were referred from the private practices of physicians in New York City. An attempt was made to select only those psychoneurotic patients without known organic disease who manifested symptoms charteristic of the early stages of adrenal insufficiency (such as asthenia and excessive fatigability, mental lethargy, lowered blood pressure, gastro-intestinal disturbances, underweight and lowered sexual drive). The formal diagnoses were neurasthenia, anxiety state and anxiety hysteria. The outstanding syndrome was chronic fatigue with mental and physical lethargy. Only those patients who manifested at least average intelligence and a high degree of cooperativeness were selected for this study.

EXPERIMENTAL PROCEDURE AND TESTS

Each patient came to the laboratory once a week for from 10 to 15 weeks. In the control tests, continued over a 3-week period, the first was intended to acquaint the patient with the experimental procedures. The next two tests were given while he was taking placebos, that is control or blank pills, without the adrenal cortex preparation, but duplicating in appearance those that contained it. The pills with the active ingredient were then given over the following weeks of the experiment. In selected cases, where the response appeared to be favorable as judged by the physiological tests, the placebos were again given so that any changes due to a sudden cessation of the treatment might be observed. Since it is well known that psychoneurotic patients often show improvement due to suggestion or to increased attention, these placebos were used as controls.

The testing procedure was carried out as follows. Each patient came to the laboratory under basal conditions and rested for one-half hour in a room free from distractions. A determination of the B.M.R. was then made with a Benedict-Roth apparatus. Following this the patient was given the Schneider Index test of neurocirculatory fitness (38). In addition, 4 psychological tests were given to a number of the patients before and at the end of the treatment. These tests consisted of: (*a*) a psychosomatic inventory of neurotic complaints (39); (*b*) a word association test (40); (*c*) a perseveration test of mental flexibility (41) and (*d*) a test for neuromuscular coordination (42).

Description of the tests. The Schneider Index of neurocirculatory fitness was developed during the World War to detect chronic fatigue and 'staleness' in aviators. It is based upon a composite score of 6 variables; the highest possible score is 18. Schneider observed that pilots who scored 7 or below were usually found to be unfit for flying because of chronic nervous exhaustion. McFarland and Huddleson (43) found that psychoneurotic patients tended to make low scores in this test.

The Index is determined by taking the pulse rate: (*a*) after reclining at least 10 minutes; (*b*) on standing; (*c*) after a standard exercise and (*d*) by

counting the time for the pulse to return to the normal standing rate. The blood pressure is also recorded while reclining and after standing, to determine whether there is a rise or fall in the systolic pressure.

The psychoneurotic inventory (39) consists of a list of psychological and physiological complaints which have been found to be more frequently experienced by psychoneurotic than by normal subjects.

The association test (40) is based upon the lists of words compiled by Kent-Rosanoff and Jung. Instead of free associations being required, each key word is followed by two other words and the subject is to indicate which of the two seems to be more closely associated in his mind with the key word. In each case one of these words is a 'normal' and the other an 'emotionalized' response.

The Maller-Elkins attention test for the measurement of perseveration (41) determines the speed and accuracy with which one can shift from solving one type of mathematical problem to solving another type. The tendency to perseverate is manifested in increased time and errors, since one mental activity tends to interfere with successive ones.

The dotting test for neuromuscular coordination is a modification of the test devised by McDougall and Smith (42) to measure mental fatigue and the effects of certain drugs.

Administration of the glycortal extract. The manufacturer's preparation of this extract is stated to be an extraction with glycerin, of the fresh adrenal glands, the whole liquid being then incorporated into gelatin-coated pills. Each pill contained 15 minims of the glycerin adrenal extract, and represented 3 grains of fresh gland substance.

The number of pills to be administered was determined by multiplying the body weight (in pounds) by two, and dividing the product by 15 (the number of minims per pill). A patient weighing 150 pounds received 20 pills a day, or approximately 7 three times a day (after meals). At the end of a week the dosage in minims was increased to three times the body weight divided by 15; that is, for a person weighing 150 pounds, 30 pills a day (90 grains of fresh gland substance). This daily dosage was maintained until the end of the course of treatment. Examination of the stools in a number of instances indicated that the substance was absorbed.

Clinical Summaries of Female Patients

L. E. Female, 28, single, Jewish, 3 years high school, stenographer (unemployed). Symptoms of fatigue and lassitude. Great effort to carry out simple tasks. Irritability and anxiety tension. Very poor social and family adjustment. No sexual urge. Diagnosis of neurasthenia.

M. C. Female, 34, married, Jewish, 1 year high school. Fatigue and exhaustion, with symptoms typical of anxiety. Headaches, insomnia and frequent hysterical spells. Nervousness and tension at home, due to difficulties with mother-in-law. Diagnosis of anxiety hysteria.

M. S. Female, 38, married, Jewish, college graduate. Easy fatigue and complete lack of energy or drive. Excessive worry over health. Mental laziness and indifference to environment. No sexual urge. Diagnosis of anxiety state.

E. B. Female, 54, widow, Protestant, college graduate. Headaches, sleeplessness, lassitude and chronic exhaustion. Excessive worry over health. Diagnosis of anxiety state.

S. R. Female, 24, single, Jewish, 1 year high school, secretary (unemployed). Frequently recurring periods of nervous tension and excitement followed by extreme fatigue and exhaustion. Spells of dizziness and mental lethargy. Excessive fears and worries about sexual adjustment. Diagnosis of neurasthenia.

G. S. Female, 44, single, Protestant, college teacher (unemployed). Fatigue and lack of energy without apparent cause. Excessive worries about health. Lack of sex drive. Diagnosis of anxiety hysteria.

C. B. Female, 48, married, Protestant, high school graduate. Fatigue and insomnia complicated by menopause syndrome. Inability to do housework or walk upstairs, because of exhaustion. Excessive physical complaints, shifting from one organ to another. No appetite and underweight. Diagnosis of anxiety state.

Clinical Summaries of Male Patients

A. R. Male, 35, single, Protestant, college graduate, teacher. Chronic fatigue, exhaustion and extreme mental lethargy. Sleeplessness, headaches and anxiety tension. Emotional maladjustment associated with sexual worries and phobias. Diagnosis of anxiety state.

J. C. Male, 56, married, Protestant, 4 years high school, male nurse. Constant general fatigue and lack of energy to work at any task. Headaches and various physical complaints without apparent organic basis. Fear of riding on subway, or entering room where there is a group of people. Diagnosis of anxiety hysteria.

S. S. Male, 25, single, Jewish, graduate student. Complete loss of self-confidence. Lack of ability to concentrate due to feeling of excessive fatigue and exhaustion. No appetite, frequent digestive disturbances and disordered sleeping. Lack of sexual urge. Diagnosis of anxiety state.

M. T. Male, 26, single, Jewish, college graduate, salesman (employed). Acute exhaustion and 'nervous collapse' with typical neurasthenic syndrome. Marked anxiety tension, weakness and loss of weight. Fears of impotence. Diagnosis of neurasthenia.

V. C. Male, 30, single, Catholic, common school, skilled laborer. Easy fatigue on exertion. Constant feeling of mental tension and uneasiness. Illness and nausea provoked by open places. Severe frontal headaches. Withdrawn libido. Diagnosis of anxiety hysteria.

H. G. Male, 45, single, Jewish, common school, salesman. Constant feeling of exhaustion. Headaches and sleeplessness. Mental laziness, indifference to his work and the environment. Intense feelings of insecurity in his personality adjustments. Sexual maladjustments. Diagnosis of anxiety state.

J. B. Male, 38, single, Protestant, high school graduate, mechanic. Excessive fatigue in mornings. No capacity for concentration. Headaches, insomnia and digestive upsets. Sexual maladjustment, no libidinal drive. Diagnosis of neurasthenia.

Results and discussion. The changes that took place in the various physiological tests during the treatment are shown in tables 1 to 4. The means of the physiological tests for the 7 female patients are tabulated in table 1, and the means for the male group in table 2. The responses of 10 of the patients who took the various psychological tests before and after the treatment are shown in table 3.

TABLE 1. THE MEANS IN THE VARIOUS PHYSIOLOGICAL TESTS BASED ON 7 FEMALE SUBJECTS

Weeks of treatment	1	2	3	4	5	6	7	8	9	10	11
Number of subjects	4	7	7	7	7	7	7	7	7	7	7
Number of glycortal pills	Control	Control	Control	19	20	24	22	23	25	25	26
Schneider Index	10.3	10.4	10.6	11.0	12.1	11.1	11.0	11.7	12.5	12.7	12.4
Pulse Rate: Reclining	62	64	65	64	65	67	64	64	60	61	62
Pulse Rate: Standing	75	78	79	71	75	80	74	74	73	72	72
Pulse Rate: After exercise	100	104	101	94	95	100	98	96	96	96	91
Pulse Rate: Time in minutes, return to normal	2.0	1.6	1.6	2.0	1.6	1.6	1.8	1.8	1.7	1.7	1.6
Blood Pressure: Reclining	109/68	112/68	113/68	114/72	112/70	115/68	118/73	123/73	118/71	122/71	118/69
Blood Pressure: Standing	108/71	110/73	111/72	112/79	116/75	115/73	117/78	124/81	121/76	125/76	120/76
Pulse Pressure: Reclining	41	44	45	42	42	47	45	50	47	51	49
Pulse Pressure: Standing	37	37	39	33	41	42	39	43	45	49	44
B.M.R.	−8.3	−9.3	−9.4	−8.3	−6.6	−6.7	−7.7	−8.6	−5.6	−6.1	−6.6
Body weight (in kg.)	57.4	56.2	56.5	57.0	57.0	56.7	57.4	57.8	57.6	57.2	56.4

Outside the objective tests, attention was given to each patient's subjective reaction to the therapy. Without discounting the suggestibility of such psychoneurotics, we have summarized these reactions below in their relation to the results of the physiological and psychological tests.

L. E. Treatment had no appreciable effect. No significant changes were observed in any of the physiological or psychological tests. Several weeks after the treatment

TABLE 2. THE MEANS IN THE VARIOUS PHYSIOLOGICAL TESTS BASED ON 7 MALE SUBJECTS

Weeks of treatment	1	2	3	4	5	6	7	8	9	10	11
Number of subjects	5	7	7	7	7	7	7	7	7	7	5
Number of glycortal pills	Control	Control	Control	27	27	32	32	35	37	37	39
Schneider Index	10	10.8	11.0	11.3	12.1	11.8	12.1	13.5	12.7	13.6	12.6
Pulse Rate: Reclining	65	63	65	61	63	63	63	63	64	63	60
Pulse Rate: Standing	79	76	75	74	75	73	75	74	77	74	71
Pulse Rate: After exercise	96	93	95	91	93	93	94	93	91	90	90
Pulse Rate: Time in minutes, return to normal	2.0	1.9	2.0	1.8	1.8	1.8	1.9	1.7	1.6	1.8	1.8
Blood Pressure: Reclining	110/68	107/62	112/67	111/66	112/64	113/67	113/63	113/68	115/68	116/67	114/67
Blood Pressure: Standing	108/72	108/71	114/73	110/75	113/72	116/75	116/72	118/75	117/72	119/71	117/72
Pulse Pressure: Reclining	42	45	45	45	48	46	50	45	47	49	47
Pulse Pressure: Standing	36	37	41	35	41	41	44	43	45	48	45
B.M.R.	−8.4	−5.0	−4.0	−2.3	−2.9	−0.7	−2.6	−1.4	0.1	0.4	−3.0
Body weight (in kg.)	67.0	68.4	68.0	68.5	68.5	68.5	68.5	68.9	69.3	68.9	70.1

TABLE 3. RESULTS OF THE PSYCHOLOGICAL TESTS FOR 5 MALE AND 5 FEMALE PATIENTS

Patient	Psychosomatic inventory[2]			Coordination test[3]	Perseveration test[4]	Association test[5]
	Physiological	Psychological	Total			
			Females			
L. E.						
Before[1]	−55	−46	−101	147	56	59
After[1]	−85	−33	−118	140	44	55
M. C.						
Before	−72	−64	−136	145	58	37
After	38	17	55	153	64	19
S. R.						
Before	−74	−83	−157	172	48	19
After	−78	−98	−176	170	40	41
G. S.						
Before	48	107	155	172	64	24
After	68	108	176	180	72	16
C. B.						
Before	44	77	121	148	38	19
After	41	102	143	159	40	8
Means						
Before	−22	−2	−24	157	53	32
After	−3	19	16	158	52	28
			Males			
J. C.						
Before	−45	−69	−114	162	44	19
After	−8	2	−6	160	48	13
M. T.						
Before	−64	−103	−167	168	52	31
After	20	−10	−20	188	56	22
V. C.						
Before	−15	−32	−47	183	68	26
After	10	18	28	179	65	33
H. G.						
Before	−55	−46	−101	179	54	38
After	−85	−13	−98	171	50	21
J. B.						
Before	57	63	110	180	64	45
After	116	117	233	198	66	35
Means						
Before	−24	−37	−62	174	56	32
After	11	23	33	179	57	25

[1] *Before* indicates response previous to the therapy; *after*, the response during the last week of the therapy.

[2] The greater the neurotic tendency, the lower the score, below −10 being suggestive of physiological dysfunction and 10–25 of psychological maladjustment.

[3] In the dotting test, the higher scores are better.

[4] In the perseveration test, the higher scores indicate less perseveration.

[5] In the association test, the lower scores indicate fewer abnormal responses.

began the patient reported that she felt less fatigued, but this feeling was not sustained. There was a slight improvement in sexual desire.

M. C. The patient reported that after the treatment she could think more clearly and take more interest in things. She stated, "I don't notice that terrible pressing in my throat or the heavy feeling in the lower part of my chest." She thought of suicide less frequently and was relieved of the extreme nervous tension. The improvement in mental behavior was reflected in the better scores on psychological tests. The Schneider Index was higher. When the treatment was discontinued most of her neurotic complaints reappeared.

M. S. The patient stated that she felt better and was less exhausted and fatigued. There was a distinct improvement in libidinal drive. Changes in the various physiological tests were slight but favorable.

E. B. The subjective effects of the treatment were favorable, in that the patient felt less exhausted and slept better. The findings in the various physiological tests showed favorable minor changes.

S. R. The subjective response was unfavorable, for at the end of the treatment this patient complained of frequent headaches, disturbances of menstruation, constipation, dizziness and fatigue. The changes in the physiological and psychological tests during the treatment were not significant.

G. S. This patient felt that the therapy diminished her feelings of bodily fatigue and the frequency of headaches and rhinitis. There was a significant, though intermittent, improvement in the Schneider Index. Before the treatment she fainted twice upon standing during the recording of the Schneider Index. This did not recur after the treatment had been in progress over 4 weeks. Psychological tests showed slight but uniform improvement.

C. B. The response of this patient to the treatment was favorable. She reported that her outlook on life was better, that she was not so 'nervous' and was able to work. In the physiological tests there was a significant increase in the Schneider Index, systolic blood pressure and body weight. The scores in the psychological tests showed improvement.

A. R. There were no significant subjective changes in this patient's condition during or following the treatment. There was an increase in the systolic blood pressure and in the B.M.R.

J. C. This patient's response to the treatment was favorable. He was able to work again and felt distinctly less fatigued. In the physiological tests his Schneider Index increased, as well as the B.M.R. He improved in 3 of the 4 psychological tests.

S. S. The subjective response of this patient to the therapy was fairly satisfactory. Although he did not completely recover from his phobias, he was able to work again and felt less exhausted. His Schneider Index was maintained at a high level and there was a rise in basal metabolism.

M. T. This patient felt much better after the treatment. There was a marked improvement in his environmental situation, however, which contributed greatly to his recovery. There was a significant increase in the scores in the Schneider Index, with a rise in basal metabolism and in weight. All the psychological tests showed scores significantly improved.

V. C. During the treatment there was improvement in his state of anxiety. His intense headaches remained. The results of the physiological tests showed a slight improvement. He had fewer neurotic complaints as judged by the psychosomatic inventory, but the other psychological tests reflected a slight retrogression.

H. G. Complaints of this patient were unaffected by the therapy. His severe exhaustion and fatigue continued. There were no significant or consistent changes in the physiological or psychological tests.

J. B. There were no significant changes in the patient's condition at the end of 4 weeks' treatment. At the end of 6 weeks, however, he reported that he felt much better, that he had fewer headaches and that his fatigue on rising was only slight. He began to work at his trade again fairly successfully. By the eleventh week the Schneider Index had risen from 10 to 14. The B.M.R. rose from −9 to +12. The scores made on all the psychological tests showed less neurotic behavior at the end of the treatment.

Because of the small number of subjects in each group, the differences between the means in the various physiological and psychological tests before and after the course of therapy were not treated by the usual statistical pro-

TABLE 4. NUMBER OF PATIENTS RESPONDING MORE FAVORABLY (POS.), WITHOUT CHANGE (UNAFF.), AND LESS FAVORABLY (NEG.), TO CERTAIN TESTS, AFTER GLYCORTAL THERAPY

Test	Criterion of change in response	Number of cases		
		Pos.	Unaff.	Neg.
		(14 subjects)		
Patients' reports of improvement (estimated)		7	4	3
Schneider Index	Rise (or fall) of 2 or more (points)	9	5	0
Pulse rate reclining	Fall (or rise) of 4 or more (beats per second)	4	8	2
Pulse rate standing	Fall (or rise) of 4 or more (beats per second)	5	6	3
Pulse rate after exercise	Fall (or rise) of 5 or more (beats per second)	8	5	1
Syst. blood press. reclining	Rise (or fall) of 5 or more (mm. Hg)	10	3	1
Syst. blood press. standing	Rise (or fall) of 5 or more (mm. Hg)	10	3	1
Pulse pressure reclining	Rise (or fall) of 8 or more (mm. Hg)	6	7	1
Pulse pressure standing	Rise (or fall) of 8 or more (mm. Hg)	10	2	2
Basal metabolic rate	Rise (or fall) of 8 or more (per cent)	8	4	2
Body weight	Rise (or fall) of 1 or more (kg.)	7	7	0
		(10 subjects)		
Inventory—physiological complaints	Rise (of fall) of 20 or more (points)	6	2	2
Inventory—psychological complaints	Rise (or fall) of 20 or more (points)	7	3	0
Coordination	Rise (or fall) of 5 or more (contacts on revolving disk)	5	3	2
Perseveration	Rise (or fall) of 4 or more (errors)	4	3	3
Word association	Fall (or rise) of 5 or more (words)	7	1	2

cedures for the reliability of the observed differences. Upon examination of tables 1, 2 and 3, one observes that the average changes found by the various tests, with certain exceptions, are hardly greater than the experimental error. Several favorable tendencies do appear to be significant if one follows the results throughout the course of the therapy.

The Schneider Index was increased in every case but one, the average change for all 14 subjects being +2.5 points. In 8 subjects, 4 female and 4 male, the pulse rate after exercise was reduced by from 6 to 18 beats per minute (cf. tables 1, 2 and 4—1. 5.). The systolic blood pressures, on reclining and on standing, showed average increases of 6 and 10 mm. Hg, respectively, for the females; of 8 and 9 for the males. Pulse pressures, on standing, gained an average of 7 mm. Hg for the females; 9 for the males.

Changes in the B.M.R. of females ranged from +8 to −12, averaging −1. In males a rise always occurred, averaging +9. Both female and male patients increased approximately 1 kg. in body weight.

In the various psychological tests, the differences between the means at the beginning and at the end of the treatment, as in table 3, are not significant, excepting those for the psychosomatic inventory. This test demonstrated that the subjective complaints of the patients, on the average, were less pronounced after the course of treatment than before it.

In an attempt briefly to summarize the response of the patients to the therapy, a table has been prepared (table 4) indicating the number whose responses to the various tests might be characterized as positive, unaffected and negative. All 14 of the patients were given the physiological tests, while only 10 were given the psychological ones.

SUMMARY

Glycerinated adrenal cortex (glycortal pills, Schieffelin & Co.) was administered by mouth to a series of 14 patients suffering from psychoneuroses marked by asthenia. The experimental period for each patient, lasting from 10 to 15 weeks, began without medication for one week, followed by two weeks of placebos or blank pills (containing no cortical extract). These weeks, and occasionally a blank-pill week later, served as controls. The increasing dosages ranged from 15 to 48 pills daily, each pill representing 3 grains of fresh gland.

The Schneider Index (of neurocirculatory fitness), B.M.R., and weight were taken weekly for all 14 patients. A battery of physiological and psychological tests was given at the beginning and end of the experimental period to 10 of the 14.

Half the patients reported subjective improvement. Half gained a kilogram in weight. The B.M.R. was accelerated (improved) in 8. The Schneider Index was bettered in 9 and unaffected in 5.

The inventories of psychological and of physiological complaints showed improvement respectively in 7 and 6 of the 10 patients so tested. The word association test indicated fewer neurotic responses in 7, neuro-muscular coordination was improved in 5, and perseveration was lessened in 4.

Following oral medication with adrenal cortex, the objective tests for improvement in asthenic psychoneurotics gave favorable results, on the average, in over half the series. We conclude that this therapy has proved valuable in a category of obstinate disability.

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THE SIZE OF THE SPLEEN AND THE ADRENALS DURING PREGNANCY AND THE PUERPERIUM

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IT WAS SHOWN by Barcroft and his coworkers (1) in 1925 that the spleen is capable of serving as a reservoir for red blood corpuscles. It is believed that when the proper stimulus arises the spleen may contract and thus augment the number of circulating red blood cells. It is estimated that about one-third of the total number of red blood cells of the cat can be stored in the spleen.

In 1928, Barcroft and Stevens (2) found that during pregnancy, the exteriorized spleen of the dog became smaller. Barcroft later (3) showed that the pregnancy contraction could be abolished by denervation, and also noted that after parturition the spleen is contracted for a greater or less period whether denervated or not. As long as 2 weeks was necessary for the spleen to return to its normal size after parturition in some cases.

It is commonly stated that the adrenals hypertrophy in pregnancy. DeLee (4), Williams (5) and Schumann (6) in their respective textbooks of obstetrics state that there is hypertrophy of the adrenal glands during pregnancy, and that the cortex is most involved. Guthmann and Voelcker (7) observed in white mice that pregnancy produced a noticeable increase in the size of the adrenals, and that the enlargement of the cortex was greater than that of the medulla. Blumenfeld (8), studied the adrenals from 176 Wistar albino rats and found that the first pregnancy in normal animals did not alter the weight of the cortex or the medulla of the adrenal gland. Animals maintained on a vitamin E deficient diet during pregnancy, however, showed cortical hypertrophy and medullary atrophy.

For the past several years, the authors have been investigating organ weights in pregnant and puerperal animals (9), and have accumulated a large volume of data which may be of considerable interest. We have been especially interested in the changes in the size of the organs immediately following parturition, a time when important readjustments must be made by the maternal organism.

METHODS

Normal female guinea pigs were selected for maturity and for large size. Only animals weighing more than 480 gm. were used. They were kept in clean, roomy cages and were fed an adequate diet. Their weight was watched carefully, and when they had reached a good state of nutrition and hydration they were weighed, then stunned by a blow on the head. The vessels of the neck were then severed and the animal was allowed to bleed to death. After the animal was exsanguinated the heart, adrenals and spleen were removed, in the order named. This precaution was taken to assure that the spleen

should have expelled all possible blood in response to the sudden blood loss. The spleen and adrenals were dissected free of adipose tissue and vessels, washed briefly in running water to free them of blood, and blotted dry at once with filter paper. The spleen and the adrenals were then weighed separately to the nearest 3 mg. The data are expressed here as the number of grams of organ substance per kilogram of body weight.

A group of pregnant animals was allowed to give birth to their young. Seventy-two hours after parturition these animals were killed in the manner outlined above, and their organ weight-body weight ratios determined in an identical manner.

In a third group of animals, pregnancy was allowed to proceed normally for 7 or more weeks. (The normal gestation period is 9 weeks.) These animals were then subjected to the same procedure as previously outlined, and their organ weight-body weight ratios determined in the same manner. The weight of the uterine contents of each of these animals was determined, and was subtracted from the total body weight. It was believed necessary to do this in order to make this group conform more closely to the controls.

The technic for determining the organ weight-body weight ratios which had been established in the controls was adhered to rigidly throughout the work.

RESULTS

Table 1 shows the results obtained regarding the changes in the spleen during pregnancy and the puerperium.

TABLE 1. SPLEEN WEIGHT-BODY WEIGHT (Sw/Bw) RATIOS DURING PREGNANCY AND PUERPERIUM

Condition	No. of animals	Average body wt. gm.	Sw/Bw Ratio gm./kg.	% change	'p'[1]
Normal	52	580	1.68		
Pregnant	33	595	1.42	−15.5	<0.01
Postpartum	29	645	1.71	1.6	>0.9

[1] 'p' refers to the probability of the difference occurring by chance, and is calculated according to Fisher (10). A 'p' of 0.05 or less indicates a significant difference.

Thus it is seen that the figure for the Sw/Bw ratios of the pregnant animals differ very significantly from the normal, while there is virtually no difference at all between the normal and the postpartum spleens. Table 2 shows the changes noted in the adrenals.

TABLE 2. ADRENALS WEIGHT-BODY WEIGHT (Aw/Bw) RATIOS DURING PREGNANCY AND PUERPERIUM

Condition	No. of animals	Average body wt. gm.	Aw/Bw Ratio gm./kg.	% change	'p'[1]
Normal	52	580	0.670		
Pregnant	33	595	0.719	6.8	0.3
Postpartum	29	645	0.899	34.2	<0.01

[1] 'p', see note at foot of table 1.

It is seen, therefore, that there is no significant increase in the size of the adrenals during pregnancy, but that there is a highly significant increase in size in the early puerperal period.

DISCUSSION

It is to be noted that great care was taken to assure that the animals used in this work were of the same statistical population and that other conditions were well controlled.

It is seen that during the antepartum period the spleen is significantly smaller than normal. This finding is in accord with the findings of Barcroft and Stevens (2). Barcroft also believes that the decrease in the size of the spleen during pregnancy is for the purpose of supplying more blood to the genital organs, or aiding in fetal hematopoesis, or both (11). Since our work, using different methods, confirms Barcroft's original work, it appears that it is well established that the spleen is definitely smaller during pregnancy in the species of animals studied thus far.

Barcroft states (3) that the spleen is contracted for some time after parturition in the dog. In the guinea pig, as studied here, it apparently is of normal size 72 hours after parturition. Our method did not allow us to determine whether there was a previous contracted phase, or whether there might be such a phase with an onset later than 72 hours after parturition.

Several textbooks of obstetrics (4, 5, 6) state that there is definite hypertrophy of the adrenals, especially the adrenal cortex, during pregnancy. However, as was pointed out in the introduction, some evidence has been presented which questions the validity of this statement, especially as regards animals normal in all other respects. There is, according to our figures, a very slight tendency toward enlargement of the adrenals during pregnancy, but it is so far from statistical significance that it is undoubtedly meaningless. It is possible to assume that the cortex might hypertrophy at the expense of the medulla, but as this does not seem likely, no attempt was made to weight the 2 parts separately. It was felt that hypertrophy could be determined more accurately by weighing the intact organ than by an attempt to separate the cortex from the medulla.

The parturient animals, however, had adrenals significantly larger than the controls. There was an increase in Aw/Bw ratio of 34.2%, and the probability of chance occurrence of this difference was far less than 1 in 100 (Fisher). We have been unable to find any mention of this phenomenon in the literature. Such a rapid change in the weight of an organ can best be explained by fluid distribution. Seventy-two hours is certainly too short a time for hypertrophy or hyperplasia of the adrenals to occur. It is definitely known that pregnancy is associated with a hypervolemia. The blood loss during parturition cannot compensate for this increase in blood volume. Therefore, following parturition there must needs be a redistribution of fluid. It seems likely to the authors that the adrenals are heavier in the puerperal state because they have, probably, along with other organs, shared in this fluid redistribution.

SUMMARY

Fifty-two normal female guinea pigs were killed by blows on the head followed by exsanguination while in a good state of nutrition and hydration. The spleens and adrenals were removed and the spleen weight-body weight (Sw/Bw) and adrenal weight-body weight (Aw/Bw) ratios determined. Thirty-three pregnant animals were killed in an identical manner after the seventh week of pregnancy. (Normal gestation period is 9 weeks.) Twenty-nine animals were subjected to an identical procedure 72 hours after parturition. The organ weight-body weight ratios of these 2 groups were calculated in the same manner as the controls. With the exception of the experimental conditions, all 3 groups of animals were of the same statistical population.

The spleen was about 15% smaller in the pregnant than in the normal animals. This finding corroborates the work of Barcroft. The spleens in the postpartum group, however, were not significantly different in weight from the controls.

The adrenals were of approximately normal size during pregnancy. Thus we do not substantiate the statement appearing in several commonly used textbooks of obstetrics that the adrenals hypertrophy in pregnancy. During the postpartum period, however, the adrenals increased in size by about 34%. The possibility of chance occurrence of this difference is less than 1 in 100.

It is suggested that the rapid increase in the weight of the adrenals in the postpartum period may be due to the changes necessitated by the redistribution of the fluid produced by the hypervolemia of pregnancy.

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THE ASSAY OF CORTICAL HORMONE

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HARROP, SWINGLE AND PFIFFNER and their coworkers (*Science* 73: 683, 1933; *J. Biol. Chem.* 104: 701. 1934) have suggested a method for assay of adrenal cortical hormone, which is based upon non-protein nitrogen estimations in the blood of adrenalectomized dogs. A certain dose of hormone which maintains the N.P.N. in the blood at its normal level is administered to adult adrenalectomized dogs. This dose is reduced at intervals of 7 to 10 days until the blood N.P.N. rises above 50 mg.%. The potency of the hormone extract is expressed in terms of dog units (D.U). A dog unit is the minimum dose per kilogram of body weight necessary for the maintenance of a dog for a period of 7 to 10 days without loss of body weight and elevation of N.P.N. level of the blood. There are the following objections to be made against this method of assay.

(*a*) The period of assay is too short because some dogs seem to be in good condition on an inadequate dose for 2 to 3 weeks, but thereafter show symptoms of insufficiency.

(*b*) Usually the dogs are not killed at the end of the assay in order to prove the absence of accessory tissues.

(*c*) The assay is complicated by the administration of cortin and sodium chloride together.

(*d*) The N.P.N. is not always a true criterion of the condition of the dog. In severe insufficiency the N.P.N. may show slight or no increase. Even a N.P.N. of 120 mg.% may occur in a dog which is in good condition.

(*e*) The adrenalectomized dogs are very susceptible to any loss of blood. Often they will die suddenly some hours after the small sample of blood necessary for the N.P.N. estimation is taken.

(*f*) The material is fairly small and the test therefore not very reliable.

In this laboratory cortin has been tested for 2 years on 2-stage adrenalectomized dogs and from our observations it is apparent that the assay is as reliable when based only on clinical symptoms as when based on chemical changes in the blood. The objections mentioned above may be met with the following.

(*a*) The choice of the period of assay is arbitrary. For example: over a period of 7 days a daily 5 cc. dose of cortin was given. The dog showed no sign of insufficiency. Then 2.5 cc. was injected and the dog became insufficient the next day. Five cc. was considered as producing a positive reaction and 2.5 cc. a negative reaction. However, if we had also injected 5 cc. on the 8th day the appetite would have been decreased that day and we would have considered the same dose of 5 cc. as producing a negative reaction. In the

first case 2.5 cc. of cortin was injected on the 8th day and a negative reaction was produced because the dog was incipiently insufficient and became insufficient the next day. At the end of this time 5 cc. was given and the dog did not recover. Ten cc. was given the next day and the dog recovered. Five cc. was considered to produce a positive reaction and 2.5 cc. a negative reaction.

If a period of 4 days is used and 5 cc. is given per day, the dog shows no insufficiency symptoms. If 2.5 cc. is given per day for 4 days, on the 3rd day the dog is insufficient. The result is the same; 5 cc. produces a positive reaction, 2.5 cc. a negative reaction. If a period of 10 days is used it will appear on the 8th day that 5 cc. produces a negative reaction and 10 cc. a positive reaction. If a period of 20 days is used 10 cc. will produce a negative reaction. The longer the period, the greater the chance that a dose will at some time produce a negative response when the dog, through unknown extraneous factors, needs more cortin. The result of the assay indicates much too large a dose and as the maintenance dose is searched for too much cortin is used. Therefore we consider that a period of 4 days is sufficient.

(*b*) The second objection raised may be an advantage. Tests for several years on the same dog furnish better material for comparison than when new dogs are used each time. In the past few years, accessory tissue has not been found in the 20 dogs used for the assays in this laboratory.

(*c*) The third point is still a difficulty, for the potency of an extract cannot be expressed in terms of dog units when sodium chloride is given by mouth since under this condition the maintenance dose of cortin bears no relation to the body weight of the dog.

(*d*, *e*) As N.P.N. estimations are of little importance to the assay, points *d* and *e* are not considered.

(*f*) Objection *f* is not answered without difficulty. The method of assay has been influenced by the experience obtained during the latter years and the possibilities of the assay of different extracts. An assay is only reliable when a certain extract is used as a standard, a certain amount of which has the same effect as a certain amount of an unknown extract. No guarantee of constancy is given when the dog unit or rat unit as determined by various methods of different investigators is mentioned in an assay of cortical extract. Indeed the same dog may require quite different doses for one and the same reaction on two different occasions, let alone different dogs.

A standard extract can be used in various ways. A dose-effect curve can be made by means of the standard extract. As the same dose of cortin does not always have the same effect, this method should be rejected. It is more useful to determine the limit of the effect of the standard and the unknown extract and to check this by determination of the inactivity of a smaller dose and the higher activity of a larger dose.

It is desirable to determine the limiting dose on a large number of animals and to choose as a standard norm the average dose which when given to the animals just maintains them. In the determination of the adequate dose of the unknown extract a cross test with two series of animals is more reliable. In such an assay the number of animals to be used cannot be fixed a priori.

The experiments with changing concentrations have to be repeated over and over again until the conformity of the unknown and the standard extract is well established. Though the principles of the ideal assay are quite obvious to us an exact assay is impracticable and with the number of dogs available in this laboratory, impossible. If one is aware, however, of the state of affairs the limited reliability of the data obtained from as few as 4 dogs is obvious. The error may amount to many times 100%.

METHOD

Healthy dogs of mongrel breed, 1 to 2 years old, of 6 to 18 kg. weight, were adrenalectomized in 2 stages (*Endocrinology* 22: 497. 1938). A few days before the operation arecoline was administered to them as a vermifuge. Their diet consisted of:

5000 gm. barley meal	150 gm. tomato juice
3800 gm. horse meat	15 cc. vitamin D oil (Organon)
1200 gm. beef drippings	84 gm. salt mixture

The salt mixture contained:

NaH_2PO_4	190 gm.	$MgSO_4$	270 gm.
$CaHPO_4$	130 gm.	Fe reductum	10 gm.
$CaCO_3$	130 gm.	$MnSO_4$	100 gm.

Potassium salts were omitted. In the first year of the cortin assays 10 kg. of food contained 100 gm. of salt mixture and in addition to the above mentioned salts, 175 gm. KH_2PO_4 per 1000 gm. was added. In the course of the investigations the dogs appeared to use half as much cortin when no potassium salts were added to the food. Sodium chloride was administered separately by stomach tube. Each adrenalectomized dog was given daily after the second operation, 0.3 gm. NaCl per kg. of body weight. Some dogs drank the salt in 200 cc. of milk, but most of them refused to drink salted milk and therefore it was given in 50 cc. of milk by stomach tube. The sodium chloride can not be given in water because, given in that form, it acts as an emetic, especially in the case of insufficient dogs.

Before the second stage of the adrenalectomy the dogs were given 80 calories per kg. of body weight of food and as soon as they were bilaterally adrenalectomized this amount was reduced to 60 calories because the dogs gained too much in weight with larger amounts of food. Apparently the basal metabolism in adrenalectomized dogs is lowered. Five hundred cc. of water and 200 cc. of skim milk were given separately each day. The food conditions were arbitrarily set up.

The amount of potassium[1] in water-containing substances was as follows: meal, 227–285 mg. %; meat, 270–272 mg.%; salt mixture, 268–274 mg.%. Skim milk is known to contain 200 mg. in 100 cc. Four hundred mg. of potassium daily was given in milk and 50 mg. in food per kg. of body weight. At the end of the investigation some attempts were made to diminish the potassium content of the food. As there seemed to be little difference in cortin requirement when 50 or 200 cc. of milk was given, the milk was left

[1] Potassium was estimated by J. Thomassen.

in the diet because it was a convenient food which was taken by insufficient dogs when they refused solid food. When for unknown reasons a dog used large amounts of cortin and sodium chloride for some length of time and still remained insufficient, 5 gm. of sodium citrate was given per day, either in milk or in water or milk by means of stomach tube.

Assay. Four symptom complexes are apparent when the test is based on the following.

(*a*) Good condition, no insufficiency symptoms
Body temperature normal, 38° to 39° C. Normal appetite, 60 calories per kg. of body weight consumed.
Positive water balance, i.e. more fluid taken up than excreted as urine.

(*b*) Slight insufficiency
Temperature subnormal, 37° to 37.9° C. Appetite slightly diminished, part of the food refused.

(*c*) Insufficiency
Body temperature, 36° to 37° C. All or nearly all the food refused. Negative water balance. Vomiting, diarrhea, adynamia.

(*d*) Severe insufficiency
Body temperature may fall to 34° to 36° C. All food refused. Negative water balance. Vomiting and diarrhea. Dog prostrated. Lowered pulse rate. Gingiva cold.

In most of the dogs the first symptom of slight insufficiency was partial refusal of food. Subnormal body temperature was not always evident. In some dogs the refusal of food began only when the temperature was below 37° C. Those dogs with a temperature of 37° to 37.9° C., and with a normal appetite were slightly insufficient. When the temperature was 37.5° to 38° C. and all food was refused the dog was considered as slightly insufficient. When the temperature was lowered to from 37° to 37.5° C. and vomiting occurred the condition was considered to be one of insufficiency.

In the assays the larger dose, that can be assumed to be positive, was always first used. For example a dog of 10 kg. weight was in good condition with 2 cc. of a control extract; the extract to be tested was injected in a similar dose which was expected to be positive. If this dose proved to be sufficient after 4 days ½ this dose was injected for 4 days, then ¼ the dose and so on, until the dog refused all food. When the body temperature remains normal the same dose is repeated until the dog refuses all food and then the dose is increased until the insufficiency symptoms vanish. When the temperature falls immediately no time must be wasted in injecting a potent dose, otherwise the dog may become instantaneously severely insufficient and die. If the first dose of the unknown extract, which was expected to be potent, was not sufficient and the dog partially refused food, twice this dose was given on the next day and was increased until the maintenance dose was reached.

The time required for the assay of an extract ranges from 9 to 17 days, an average of 12 days.

We have, at the time of writing, in this laboratory, the following dogs that have been adrenalectomized and are still being used for test purposes: 1 dog which has been in use for more than 3 years; 5 dogs which have been in use for more than 9 months; 1 dog, more than 8 months; 3 dogs, from 4 to 6 months and 2 dogs, 3 months.

The intra-individual variations in testing appeared to be larger than the

variation between various extracts of cortin (cortin was supplied by Organon, Oss, The Netherlands). As cortin is always manufactured by the same process this is not astonishing. The following data show the variations between tests upon the same dog.

Period 1. The dogs were treated with a special salt mixture, 7.8 mg. per kg. of body weight of potassium (as KH_2PO_4) per day was given.

Dog No.	Dose of cortin
89	10 cc.+; 5 cc.−; 5 cc.+; 2½ cc.−; 10 cc.+; 5 cc.−
124	15 cc.+; 10 cc.−; 15 cc.+; 10 cc.−; 20 cc.+; 10 cc. doubtful
64	2½ cc.+; 1 cc.−; 5 cc.+; 2½ cc.−
127	5 cc.+; 2½ cc.−; 1 cc.+; ½ cc.−
133	5 cc.+; 2½ cc.−

Period 2. No KH_2PO_4 was added.

Dog No.	Dose of cortin
89	2½ cc.+; 1 cc.−
124	10 cc.+; 5 cc.−
64	5 cc.+; 2½ cc.−; 1 cc.+; ½ cc.−; ½ cc.+; ½ cc.−
127	½ cc.+; ¼ cc.−
126	1 cc.+; ½ cc.−; 1 cc.+; ½ cc.−
136	2 cc.+; 1 cc.−; 2 cc.+; 1 cc.−
137	¼ cc.+; ⅛ cc.−; 1 cc.+; ½ cc.−; 1 cc.+; ¼ cc.−
138	¼ cc.+; ⅛ cc.−; 1 cc.+; ½ cc.−; 2 cc.+; 2 cc.+; 1 cc.−

+indicates a positive reaction −indicates a negative reaction

These data give the impression that after the omission of KH_2PO_2 from the salt mixture dogs *64*, *89*, *124* and *127* needed half as much cortin.

Water balance. The water balance was calculated daily for 17 dogs. When the fluid intake within 24 hours was larger than the fluid excretion, the dog was considered as in positive water balance. The fluid intake was calculated from the amount of water taken up with the diet, milk and drinking water (the diet contained 33% and the skim milk 90% water). The water balance was of little value for the cortin assay because of very large daily variations.

Normal or adrenalectomized dogs in good condition were never observed to be in negative water balance. If a dog is found in negative water balance on a certain day, one may assume that this dog is more or less insufficient or will be so very soon. Table 1 shows the data of a large number of observations. These data are calculated per 10 kg. of body weight. The table shows the number of dogs, the number of observations, the weight of the dogs together in kg., the amount of water that was taken up from milk, from food and from drinking water per day per 10 kg. and further the percentage of urine in relation to the amount of total water taken and the percentage of urine in relation to the amount of water taken up from water and milk. The latter data show that the amount of urine of normal dogs and of dogs with one adrenal left intact, whether the incision is healed or not, is 54 to 60% of the amount of water taken up from milk and drinking water, independent of the extraneous temperature.

After the administration of 0.3 gm. of sodium chloride per kg. of body weight to these dogs, the amount of urine increased to 72 to 78%. In adrenalectomized dogs in good condition and those recovering from insufficiency the amount of urine was 59 to 60%, although sodium chloride was given, and is therefore lower than in normal animals.

Experimental Conditions	No. of dogs	No. of determinations	Weight	Water taken up per 10 kg.				Urine in cc. excreted per 10 kg. dog	Urine in % of water taken up totally	Urine in % of water taken up by water and milk
				in milk	in food	in water	total			
			kg.	cc.	cc.	cc.	cc.			
Normal−NaCl	3	37	30	180	71	80	341	158	46	61
One adrenal−NaCl	6	109	78	138	68	188	394	177	45	54
One adrenal−NaCl, wound not healed	8	123	92	158	68	144	370	170	46	56
One adrenal−NaCl, high environm. temp.	3	25	47	108	64	200	372	167	46	54
Normal+NaCl	2	74	17	210	70	326	606	387	64	72
One adrenal+NaCl	3	36	45	120	65	220	405	257	63	78
adrenalectomized dogs										
Good+cortin +NaCl	17	1056	203	150	68	290	508	254	50	58
Good−cortin +NaCl	12	100	137	169	67	202	438	255	58	69
Good−cortin −NaCl	2	9	25.5	141	63	224	428	282	66	77
Slight insuff. +cortin+NaCl	17	306	203	131	31	264	426	249	58	63
Slight insuff. −cortin+NaCl	12	135	147.5	108	23	210	341	225	66	71
Slight insuff. −cortin−NaCl	6	28	64	83	22	134	239	180	75	83
Insuff.+cortin +NaCl	11	62	128	99	0.7	255	355	244	69	69
Insuff.−cortin −NaCl	4	10	51.5	47	0	49	96	100	104	104
Recovery+cortin +NaCl	15	188	188	140	38	313	491	277	56	61

After omitting cortin the percentage of urine increased in the same way when sodium chloride was omitted. The relative increase occurred in all stages of insufficiency.

In insufficiency the percentage of urine amounted to 104% that is more urine was excreted than fluid taken up, the dogs were in negative water balance.

These experiments show that in insufficiency the relative diuresis is increased and that this is the same when the dog is in good condition, when cortin or sodium chloride is omitted.

SUMMARY

The assay of cortin in dogs based upon clinical symptoms, principally appetite and body temperature is described.

The symptoms of insufficiency are sharply defined. The larger dose of extract that can possibly be assumed to be positive is tested first, then the dose is diminished until the dog becomes insufficient. The choice of assay period is arbitrary, a period of 4 days is sufficient for one dose of extract. It is necessary to determine the limiting dose in a large number of dogs and to check this dose with the limiting dose of a known standard extract. Water balance experiments are of little value for the assay, but from large numbers of estimations it is concluded that in insufficiency fluid excretion is increased in relation to fluid intake.

THE EXAMINATION OF THE BLOOD OF NORMAL AND ADRENALECTOMIZED DOGS IN RELATION TO CORTIN TREATMENT I

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RECENT INVESTIGATIONS in America emphasizing the morphological and chemical changes in the blood of adrenalectomized dogs and of patients with Addison's disease, have led us to our investigation on dogs. The blood of 6 normal dogs was studied for alterations in potassium and sodium level in the plasma, in non-protein nitrogen, in sugar and water content, and in morphological structure. Blood samples were taken 4 times from each dog. Subsequently the dogs were deprived of the right adrenal, the operation being performed under sterile conditions as described by Freud, Uyldert and Waterman (1). Again blood samples were taken 4 times from each dog and after removal of the left adrenal 6 to 9 times during various degrees of insufficiency. The degree of insufficiency was determined by clinical symptoms. The following degrees can be distinguished.

(*a*) Good condition, temperature normal, 38° to 39° C., food not refused.
(*b*) Slight insufficiency, temperature at least 1° below the normal level (37° to 37.9° C.), some food refused.
(*c*) Insufficiency, temperature below 37° C., all or nearly all food is refused, negative water balance, further symptoms such as diarrhea, vomiting, adynamia may occur.
(*d*) Severe insufficiency, same symptoms as in (*c*), dogs are prostrate.

Since it frequently happens that after deprivation of blood the dogs cannot be brought through severe insufficiency, blood samples were taken only when the dogs were in conditions (*a*), (*b*) or (*c*). The question arose whether or not the increase of potassium and decrease of sodium in the blood plasma occur as early or as late symptoms, and whether or not these changes would be useful criteria in assay of cortical hormone.

Blood chemical studies have been made by many investigators, especially in America. Changes in the chemical constituents have been investigated for the time dogs and cats live after adrenalectomy without treatment with cortical extracts. This time is about 6 days. Investigations have also been carried out on the changes in the blood in adrenalectomized animals over long periods in which cortin is administered and withdrawn (2–10).

In insufficiency the N.P.N. may increase from 5 to 6 times its normal value but some dogs die from severe insufficiency when the N.P.N. is only twice its normal value. From the work of Loeb (9) and Harrop (7) it was shown that in insufficiency sodium and chlorine are decreased by only 15% and an increased sodium chloride diuresis is involved. Partial therapy can be applied by administering sodium chloride or other sodium salts by mouth or intravenously. When sodium decreases potassium simultaneously increases. Hast-

ings and Compere (11) found 7 times more potassium in the blood of insufficient animals than in normal ones. In 1936 Allers, Wilson and Kendall (12) assumed that adrenal insufficiency was caused by increase of potassium in the blood and not by decrease of blood sodium chloride. This is the basis of the therapy, carried out by Wilder et al. 1937 (13), by means of which Addisonian patients were kept in good condition on a low potassium diet.

METHODS

The experiments were carried out with 7 dogs after right and left adrenalectomy. Four of these dogs were also studied with both adrenals intact. Three dogs were studied only with intact adrenals and 3 other dogs both before and after the removal of one adrenal. Blood samples were taken in the morning 15 to 16 hours after the last meal. It was necessary to take this precaution in order to eliminate the variable amount of K, Na and sugar taken up in the food.

The blood sample was taken from the jugular vein by the use of an oiled syringe. It was immediately transferred to an oiled centrifuge tube containing heparin as an anticoagulant. One hundred mg. of heparin was shaken with 2 cc. of distilled water and 1 drop was used for 6 cc. of blood. The centrifuge tube held 6 cc. and was closed by a rubber stopper, thus the blood was practically shut off from the air. Immediately after taking the sample, the blood was centrifuged for 15 minutes at 3000 r.p.m. The K and Na was estimated in the plasma. For N.P.N. 3 cc. of blood was taken with potassium oxalate as an anticoagulant. Another 0.5 cc. of blood was required for the blood sugar estimation and, for the plasma and cell volume estimation and morphological structure, a few drops more. Altogether 10 cc. was taken within 3 minutes. Dogs of 8 kg. body weight could not stand this treatment and collapsed some hours later, therefore dogs of 15 kg. weight were preferably used.

The N.P.N. was estimated by the micro-Kjehldal method of Parnas-Wagner (14), the blood sugar by that of Hagedorn and Jensen (15).

The estimation of potassium. The method of Breh and Gaebler (16, 17) was used. One cc. of blood plasma was digested with sulphuric acid in a silica dish. The residue was dissolved in water, placed into calibrated 15 cc. centrifuge tubes and the solution made up to 3 cc. Three control solutions were made in the same way containing 100, 250 and 400γ of K respectively. The K was precipitated by a sodium-silver-hexacobaltiate solution, prepared by dissolving 600 mg. of the purified reagent in 4 cc. of water. After adding 10% silver nitrate solution until a precipitate just appeared the reagent was made up to 5 cc. and centrifuged. To each of the potassium solutions 0.3 cc. of the reagent was added and after a thorough mixing, they were allowed to stand for 45 minutes. The supernatant fluid was taken off by suction and after washing once with 30% alcohol the precipitate was dissolved by warming in 1 cc. of 2N nitric acid and made up to 3 cc. After adding 5 cc. of a 5% alcoholic solution of ammonium thiocyanate the solution was made up with alcohol to 10 cc. The amount of potassium was checked in a colorimeter

against a standard solution of cobalt thiocyanate in alcohol. From the readings of the 3 standard solutions a curve can be constructed by means of which the amount of potassium in the blood plasma can be calculated.

The estimation of sodium. The estimation of sodium was carried out by the polarographic method of Majer (18). One-tenth cc. of blood plasma was digested by sulphuric acid in a silica dish. The residue was dissolved in water and brought into a measuring tube of 5 cc. capacity. After adding 0.5 cc. of a 0.4N solution of tetramethylammonium hydroxide, the solution was made up to 5 cc. The amount of sodium and potassium together was checked in the polarograph against a solution which contained a known amount of sodium alone. The amount of sodium in the plasma was found by subtracting 1.23 from the value found in the polarograph for sodium and potassium together.

EXPERIMENTAL RESULTS

The results of N.P.N. and blood sugar determinations are shown in tables 1 and 2. Estimations were carried out in normal dogs with and without administration of sodium chloride by stomach tube 15 to 16 hours before the withdrawal of blood. The same was investigated in dogs with one adrenal left intact and again in the same dogs after the removal of both

TABLE 1

Condition	N.P.N. in mg%			No. of animals	Calculated extremes	$\frac{\sigma}{\sqrt{n}}$	Blood sugar			No. of animals	Calculated extremes	$\frac{\sigma}{\sqrt{n}}$
	M	σ	n				M	σ	n			
Normal	29.0±	4.7	10	5	24.3–33.7	1.5	0.85±0.09		13	8	0.76–1.04	0.012
Normal+NaCl	27.0±	4.1	7	5	22.9–31.1	1.5	0.84±0.07		7	6	0.77–0.91	0.027
One adrenal left	31.0±	5.4	9	5	25.6–36.4	1.8	0.83±0.07		18	10	0.76–0.90	0.017
One adrenal left +NaCl	27.0±	3.4	4	4	23.6–30.4	2.0	0.81±0.07		17	9	0.74–0.88	0.017
Adrenalectomized good condition	57.0±	28.0	15	6	29 –85	7.0	0.76±0.08		26	7	0.68–0.84	0.016
Slightly insufficient	71.0±	28.0	9	5	43 –99	9.0	0.74±0.08		15	5	0.66–0.82	0.021
Insufficient	104.0±	9.8	5	4	94 –114	4.0	0.74±0.11		9	6	0.63–0.85	0.037

M = mean
σ = standard-deviation
n = number of estimations
$\frac{\sigma}{\sqrt{n}}$ = m = average error from M.

adrenals. Since in bilaterally adrenalectomized dogs it is usually necessary to give NaCl to maintain the animal in good condition it is desirable to compare normal animals and animals with one adrenal left intact, which are treated in the same way. The tables show that oral administration of sodium chloride given 15 to 16 hours before the withdrawal of blood has no effect on the amounts of N.P.N. and sugar in the blood.

Further, in table 1, it is shown that in normal and unilaterally adrenalec-

tomized dogs the blood N.P.N. may amount to from 22.9 to 36.4 mg.%. A value above 40 mg.% is never found. The standard deviation is not larger than 5.4. After total ablation of the adrenals the standard deviation is larger, 28, the variation being much increased. In dogs in good condition values of from 29 to 85 mg.% may occur. Three out of 7 dogs in good condition showed the same values as normal dogs. In 2 dogs the N.P.N. level was a little increased. Two dogs had a really high level without in the least showing clinical symptoms of insufficiency, though the same dogs had a normal N.P.N. level before deprivation of the adrenals.

In abduction one of the dogs showed nephritis, in the other dog no kidney or liver abnormality was noticed. It is apparent from this that the N.P.N. is not a measure of the degree of insufficiency in all adrenalectomized dogs. In slightly insufficient dogs the N.P.N. amounts to from 43 to 99 mg.% and is always above the normal level. In insufficient dogs it amounts to from 94 to 114 mg.%, with a standard deviation of 9.8, the variability being again reduced.

TABLE 2

	N.P.N.			Blood sugar		
	D	M diff.	Significant	D	M diff.	Significant
D between normal and unilateral adrenalectomized without NaCl	2	2.34	−	0.02	0.021	−
D between normal without NaCl and normal+NaCl	2	2.12	−	0.01	0.029	−
D between normal and adrenalectomized good condition	28	7.16	+	0.08	0.020	+
D between adrenalectomized good and slight insufficiency	14	11.4	−	0.02	0.024	−
D between adrenalectomized good and insufficient	47	8.06	+	0.05	0.040	−
D between slight insuff. and insuff.	33	9.84	+	0.03	0.043	−
D between normal without NaCl and adrenalectomized Insuff.	75	4.64	+	0.13	0.039	+

D=difference of two means.
M diff.=average error from D.
When $D<3\times m$, than the difference between the two means is insignificant.

Table 2 shows the following.

(a) The difference between normal and unilaterally adrenalectomized dogs with or without administration of sodium chloride is not significant.

(b) The difference between normal and 2-stage adrenalectomized dogs irrespective of their condition is significant, while the difference between slight insufficiency and insufficiency is also significant, but the difference between good condition and slight insufficiency is not significant. From this it is obvious that the sharp difference that can be made between good condition and slight insufficiency following the clinical symptoms of appetite and temperature cannot be made from observations of the N.P.N. level in the blood.

The very large variability in the N.P.N. values after the second adrenalec-

tomy in some dogs (but not all of them) is striking and makes it impossible to consider a dog with a blood N.P.N. level of 40 to 60 mg.% to be insufficient. Conversely, however, a dog showing a N.P.N. level of less than 40 mg.% is certainly not insufficient. The N.P.N. estimations in the blood are of inferior importance for the assay of cortical hormone.

Blood sugar. The blood sugar (table 1) amounts to from 0.74 to 1.04 mg.% with a standard deviation of at most 0.09 in normal and unilateral adrenalectomized dogs with or without administration of sodium chloride 15 hours before the withdrawal of blood.

TABLE 3

Condition	K and Na K in mg.%			Na in mg.%			Number of estimations	
	M	σ	mM	M	σ	mM	K	Na
Without NaCl	18.3	±2.54	±0.70	335	±19.6	±5.24	13	14
+NaCl	17.7	±3.45	±1.04	340	±23.6	±7.11	11	11
Totally	18.0	±2.94	±0.60	337	±21.1	±4.22	24	25
Unilateral adr. without NaCl	19.4	±2.85	±0.64	332	±19.3	±4.32	20	20
+NaCl	19.3	±2.24	±0.53	325	±15.9	±3.75	18	18
Totally	19.3	±2.54	±0.41	329	±17.9	±2.91	38	38
Bilat. adren. good	24.1	±3.14	±0.60	313	±15.3	±2.94	27	27
Slight insuff.	30.2	±5.21	±1.44	300	±17.9	±4.96	13	13
Insufficient bilateral adren.	32.9	±6.20	±2.34	299	±13.5	±5.09	7	7
5 cc. or more	24.5	±2.87	±0.77	306	±15.9	±4.40	14	13
2–5 cc.	24.0	±3.47	±1.00	319	±10.1	±2.92	12	12
0–2 cc.	28.0	±4.80	±1.52	301	±18.8	±5.66	10	11
1–2 cc.	26.6	±4.24	±1.73	—	—	—	6	—
0–1 cc.	30.0	±5.46	±2.73	—	—	—	4	—
No cortin	35.4	±2.58	±0.91	298	±14.4	±5.09	9	8

In 2-stage adrenalectomized dogs these values are from 0.63 to 0.85 mg.% with a standard deviation of, at most, 0.037. In table 2 it is shown that there is a significant difference only between dogs with both or one adrenal left intact and adrenalectomized dogs, whether insufficient or not. In the latter dogs there is no difference between those in good condition and those in severe insufficiency. Therefore the blood sugar test is of no use for assaying cortical hormone as the blood sugar content is not influenced by cortin.

The sodium and potassium level in the blood plasma. Table 3 shows the data concerning the K and Na content of the blood plasma in mg.% in normal dogs, both with and without oral administration of sodium chloride 15 hours before the withdrawal of blood. The following conclusions may be drawn from the data taken under the same conditions on dogs with one adrenal left intact and on dogs after ablation of both adrenals, which are given sodium chloride combined with various doses of cortin and which show various stages of insufficiency.

In normal dogs and dogs with one adrenal left intact, there is no difference in the plasma K and Na level whether NaCl is administered 15 hours before the blood sample is taken or not. The plasma K amounts to from 17.7

to 19.4 mg.%, the plasma Na to from 329 to 340 mg.%. In adrenalectomized dogs the plasma K amounts to from 24.0 to 35.4 mg.% and the plasma Na to from 298 to 319 mg.%.

Table 4 illustrates the significance of the difference between the average values found in adrenalectomized dogs against those obtained with normal

TABLE 4. K AND Na DIFFERENCE BETWEEN BILATERAL ADRENALECTOMIZED AND NORMAL OR UNILATERAL ADRENALECTOMIZED DOGS

Condition	D	K m.D.	Significant	D	Na m.D.	Significant
Adrenalect. good—normal	6.1	±0.85	+	24	±5.1	+
Adrenalect. slight insuff.—normal	12.2	±1.56	+	37	±6.5	+
Adrenalect. insufficient—normal	14.9	±2.42	+	38	±6.6	+
Adrenalect. good—unilat. adr.	4.8	±0.73	+	16	±4.1	+
Adrenalect. slight insuff.—unilat. adr.	10.9	±1.50	+	29	±5.8	+
Adrenalect. insufficient—unilat. adr.	13.6	±2.38	+	30	±5.9	+
Adrenalect—normal						
5 cc. cortin or more	6.5	±0.98	+	31	±6.1	+
2–5 cc. cortin	6.0	±1.17	+	18	±5.1	+
0–2 cc. cortin	10.0	±1.63	+	36	±7.1	+
1–2 cc. cortin	8.6	±1.83	+	—	—	?
0–1 cc. cortin	12.0	±2.79	+	—	—	?
no cortin	17.4	±1.09	+	39	+6.6	+
Adrenalect—unilat.						
5 cc. cortin or more	5.2	±0.87	+	23	±5.3	+
2–5 cc. cortin	4.7	±1.08	+	10	±4.1	—
0–2 cc. cortin	8.7	±1.57	+	28	±6.4	+
1–2 cc. cortin	7.3	±1.78	+	—	—	?
0–1 cc. cortin	10.7	±2.76	+	—	—	?
no cortin	16.1	±1.0	+	31	±5.9	+

dogs and dogs with one adrenal. This is calculated according to the clinical condition of the dogs, irrespective of the amount of cortin used for their maintenance and according to the amount of cortin given in the two previous days, irrespective of the condition of the dogs.

The difference between normal dogs or dogs with one adrenal left intact and bilaterally adrenalectomized dogs appears always to be significant irre-

TABLE 5. DIFFERENCE BETWEEN VARIOUS DOSES OF CORTIN INJECTED TWO DAYS PREVIOUS, IRRESPECTIVE OF THE CONDITION OF INSUFFICIENCY

	D	K m.D.	Significant	D	Na m.D.	Significant
5 cc. or more—2–5 cc.	0.5	±1.26	—	13	±5.3	—
5 cc. or more—0–2 cc.	3.5	±1.70	—	5	±7.2	—
5 cc. or more—no cortin	10.9	±1.19	+	8	±6.7	—
5 cc. or more—0–1 cc.	5.5	±2.84	—	—	—	?
5 cc. or more—1–2 cc.	2.1	±1.89	—	—	—	?
2–5 cc.—0–2 cc.	4.0	±1.82	—	18	±6.4	—
2–5 cc.—no cortin	11.4	±1.35	+	21	±5.9	+
2–5 cc.—0–1 cc.	6.0	±2.91	—	—	—	?
2–5 cc.—1–2 cc.	2.6	±2.00	—	—	—	?
0–2 cc.—no cortin	7.4	±1.77	+	3	±7.6	—
1–2 cc.—0–1 cc.	3.4	±3.23	—	—	—	?
1–2 cc.—no cortin	8.8	±1.95	+	—	—	?
0–1 cc.—no cortin	5.4	±2.88	—	—	—	?

spective of the condition or the cortin treatment of the adrenalectomized animals.

Table 5 shows the significance between the differences found between plasma K and plasma Na levels after treatment with various doses of cortin for 2 days previous to the withdrawal of the blood. From this there appears a significant difference between no cortin treatment and treatment with 1 to 2 cc. or more. The difference is insignificant between no cortin and treatment with 0 to 1 cc. of cortin and also between 0 to 1 cc. of cortin and higher doses.

At present the question whether or not the amount of potassium in the plasma depends on the amount of cortin administered is not yet solved. It is, however, obvious that the dose of cortin necessary to keep the normal potassium level in the adrenalectomized dogs amounts to at least 2 cc. per day. As in adrenalectomized dogs in good condition the plasma potassium level is elevated in comparison with the level found in normal dogs and in dogs with one adrenal left intact, the increased plasma potassium level may be assumed to be one of the first symptoms of adrenal insufficiency. The plasma sodium level is not altered significantly by various doses of cortin and therefore cannot be used in the assay of cortical hormone.

SUMMARY

In 7 normal dogs potassium and sodium content of the blood plasma and the content of N.P.N. and glucose in the whole blood were investigated. The blood of the same animals was investigated again subsequent to the removal of one adrenal and then again after the removal of the second adrenal. The administration by mouth of 0.3 gm. of sodium chloride per kg. of body weight 15 hours before the sample of blood was taken, had no effect upon the substances investigated.

No change in chemical constituents was found after the removal of one adrenal. A significant difference was found between normal dogs or dogs with one adrenal left intact and bilaterally adrenalectomized dogs, concerning the N.P.N. level in the blood, the blood sugar content and the level of potassium and sodium in the plasma.

A significant difference in 2-stage adrenalectomized dogs between a good condition and a slight insufficiency was found only in the plasma potassium level but not in the other substances estimated. A significant difference exists between slightly insufficient and insufficient dogs only in the N.P.N. level, but not in the blood sugar or the plasma potassium or sodium levels.

From this it is shown that the sharp difference between various degrees of insufficiency, which can be observed from the clinical symptoms of appetite and body temperature, cannot be deduced from changes in the blood chemical substances. Among the first symptoms of insufficiency showing themselves before the clinical symptoms occur, are to be reckoned an increased amount of N.P.N. and a decreased amount of sugar in the blood or increased potassium and a decreased sodium content in the plasma. However, for the practical assay of cortin, if done under experimental conditions as described, the estimation of N.P.N., blood sugar, plasma potassium and plasma sodium are of inferior importance.

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THE EXAMINATION OF THE BLOOD OF NORMAL AND ADRENALECTOMIZED DOGS IN RELATION TO CORTIN TREATMENT II

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LITERATURE on the blood picture of adrenalectomized animals is very incomplete. The older literature only describes data on the blood picture immediately after adrenalectomy, since, at that time, potent extracts from the adrenal cortex were unknown and it was therefore impossible to maintain the life of adrenalectomized animals.

No changes in the hemoglobin in rabbits after the removal of the adrenal glands were observed by Ohguri (1). Investigations using cats as the experimental animals were carried out by Zwemer and Lyons (2), Britton and Silvette (3) and Corey and Britton (4). Zwemer and Lyons found relative neutropenia and lymphocytosis. Britton and Silvette observed the typical increase of hematocrit values during insufficiency after adrenalectomy and improvement after injection of a potent extract of adrenal cortex. Corey and Britton observed concentration of the blood during adrenal insufficiency with increase in the number of erythrocytes. They also found an improvement when cortical extract was administered. The number of leucocytes did not change but a relative lymphocytosis could be demonstrated. A concentration of the blood during adrenal insufficiency of rats was found by Perez Miralles (5).

An increase in hemoglobin and of the number of red blood cells after adrenalectomy in dogs has been reported by Gradinescu (6), Stewart (7) and Rogoff and Stewart (8). This was confirmed by Viale and Bruno (9), Viale (10) and Swingle, Vars and Parkins (11). However, Von Haam and Thatcher (12) were unable to observe these changes. Viale (10) and Viale and Bruno (9) described changes in the number of leucocytes. Day et al. (13) observed an increase of plasma volume during insufficiency.

Therefore it seemed desirable to observe the complete blood picture of a number of dogs before adrenalectomy, after the first stage of the operation and after the second stage. The animals were maintained in good health after the second stage by the injection of cortin. Hence changes due to the operation as such could be excluded. When no cortin was given the dogs showed signs of insufficiency.

Since it is known that the range of normal characteristics of the blood picture is very large it is necessary to give data on the blood picture of the dogs before adrenalectomy. In Scarborough's monograph (14) on the blood picture of normal laboratory animals hundreds of counts are given. Differences of 50% are common. From this it may be concluded that the only

suitable control is the same animal before adrenalectomy. In the present investigation the following were recorded.

(*a*) Number of red blood corpuscles
(*b*) Size of the red blood corpuscles
(*c*) Hemoglobin content
(*d*) Hematocrit value
(*e*) Number of white blood corpuscles
(*f*) Differential count of white blood cells

The volume of each erythrocyte in cubic μ and the quantity of hemoglobin per erythrocyte in micrograms were also calculated. All investigations were performed by means of the usual standard methods. For the determination of the size of the red blood corpuscles a red-cell diameter distribution curve as indicated by Price-Jones was made and for each determination 1000 cells were measured. The results are given in table 1.

TABLE 1. RED BLOOD PICTURE

Condition	Hematocrit	Erythrocytes no.	Erythrocytes vol.	Erythrocytes hemoglobin	Hemoglobin	Size erythrocytes in μ				No. observations
						8	7	6	5	
				Dog 136						
Before 2nd operation	53± 1,1	6.3±0,3	84±2,6	32± 1,5	20,3±0,6	30± 5	845± 15	123± 10	2± 1	3
after 2nd op. good	39± 1,3	4.9±0,1	79±2,2	31± 1,4	15 ±0,5	11± 3	816± 26	167± 19	6± 2	4
aft. 2nd op. light insuf.	37± 5	5.2±0	72± 10	33±0,4	17 ±0,3	21± 5	836± 2	141± 6	2± 1	3
aft. 2nd op. insuf.	53± 15	5.3±0,4	104± 36	32± 3	16.6±0,7	4± 2	800 44	190± 45	6± 1	2
				Dog 137						
Before 2nd operation	53± 2	7.1±0,3	77± 4	31± 1,5	21,8±0	7± 2	907± 16	84 ± 14	1± 1	3
aft. 2nd op. good	49± 5	5.7±0,6	86± 6	31±0,5	18,1± 1,4	26± 3	832± 11	142± 9	1± 1	5
aft. 2nd op. light insuf.	43± 5	5.9±0,2	72± 1,5	27±0,4	16,2± 1,3	25± 3	832± 18	141± 19	2± 1	3
aft. 2nd op. severe insuf.	47± 13	5.4±0,3	85± 10	34± 2,9	18,5± 2	28± 3	814± 8	157± 11	3± ½	3
				Dog 138						
Before 2nd operation	45± 1	5.9±0,1	76± 5	31± 2	18,2±0,9	36± 5	881± 1	82 ± 6	½± ½	3
aft. 2nd op. good	41± 4	5.6±0,3	75± 11	30± 1.5	15,3±0,9	32± 4	858± 11	110± 10	2± ½	6
aft. 2nd op. light insuf.	44± 2	5.5±0,4	80± 4	30± 1,4	17 ±0,7	33± 5	842± 8	121± 13	3± ½	5
aft. 2nd op. severe insuf.	46	6.6	70	26	17,4	23	860	115	2	1
				Dog 140						
Before operation										
after 1st operation	38± 2,3	6.5±0,2	58± 4	26± 1,7	16,5± 1	28± 5	885± 1	85 ± 7	5± 1½	3
aft. 2nd op. good	41± 1,4	6.1±0,2	68± 1.5	30± 2	17,7±0,6	14± 9	745± 24	217± 15	24± 9	3
aft. 2nd op. severe insuf.	39± 1,7	5.4±0,3	70± 1,4	28±0,5	15 ±1,1	16± 3	791± 4	189± 6	3± 1½	4
	54	6.8	80	28	19,1	9	763	216	12	1
				Dog 146						
After 1st operation	44± 2,6	5.9±0,3	75± 3	30±0,8	17,6±0,7	30± 9	854± 6	115± 11	1½± 1	4
aft. 2nd op. good	45± 1,3	6 ±0,2	76± 2	29± 1,8	17,4±0,9	26± 8	845± 12	125± 15	4± ½	3
aft. 2nd op. light insuf.	46± 6	7.2±0,6	65± 1,6	26± 1,6	18,3±0,8	22± 14	829± 13	145± 27	4± 0	2
aft. 2nd op. severe insuf.	59	5	99	32	19.1	16	841	139	4	1

The variations are very large and the deviations are important. In 2 dogs the increase in hematocrit values during insufficiency could be demonstrated. This was the only value in which a difference between the normal data and those during insufficiency could be found. It is possible that the observations by other investigators were made during adrenal insufficiency just before the death of the animals. Our dogs were observed for several months and the insufficiency was not irreversible. The size of the red blood cells, the erythrocyte volume and the erythrocyte hemoglobin did not show any change. From this we conclude that the changes in the red blood cell picture are not of importance for the diagnosis of adrenal insufficiency. Clinical symptoms are very clearly shown when changes in the blood picture can not be found. The data observed on the white blood cell picture is given in table 2. We

could not confirm the findings of others that during adrenal insufficiency a lymphocytosis can be observed. No change in the leucocyte count nor in the differential count could be found in our dogs.

TABLE 2. WHITE BLOOD PICTURE

Condition	Thousands	Neutrophils %	Eosinophils %	Basophils %	Stab-neutroph. %	Juveniles %	Lymphocytes %	Monocytes %
			Dog 136					
After 1st operation	8.9± 1,4	53± 1,9	4½± 1,3	0	0,6± 0,4	0,6± 0,4	37± 1,5	6½± 2
after and op. good.	12.8± 0,7	52± 4	5± 0,9	0	1 ± 0,4	0	39± 2,4	1½± 0,4
after and op. light insuf.	12.8± 0,4	50± 4	6 ± 2½	0	3 ± 2	0	38± 6	3 ± 0,8
after and op. severe insuf.	10.1± 0,8	52± 5	4 ± 0	0	1½ ± 1	0	33± 1	4 ± 1
			Dog 137					
after 1st operation	7.4± 0,6	60± 0,8	1 ± 0,3	0	3 ± 0,4	0	31± 4	4 ± 0,2
after and op. good	117 ± 1,4	49± 0,8	3½± 0,2	0	3 ± 1,1	0	43± 2,3	2½± 1
after and op. light insuf.	9.3± 0,3	48± 0,3	10½± 0,5	0	1 ± 0,4	0	38± 2	1½± 0,8
after and op. severe insuf.	13.7± 2.3	57± 11	4 ± 2,4	0	1 ± 0,7	0	29± 10	2 ± ½
			Dog 138					
after 1st operation	12.6± 0,2	72± 1,7	4 ± 1½	0	0	0	24± 2,6	0,6± 0,1
after and op. good	12.4± 0,7	55± 4	3 ± 0,4	0	½ ± 0,2	0	39± 4	2½± ½
after and op. light insuf.	13.6± 0,7	52± 3	3 ± 1	0	0,8± 0,3	0	42± 4	3 ± 1,3
after and op. severe insuf.	20.5	70½	1½	0	4½	0	19	4½
			Dog 140					
before operation	8.3± 1,1	60± 3½	3 ± ½	0	½ ± 0,3	1± ½	31± 2	5 ± 0.2
after 1st operation	7.6	62± 4	4½± 0,9	0	1 ± 0,3	0	29± 3	2 ± 1,3
after and op. good	17 ± 2,8	60± 3	4 ± 0,9	0	0,3± 0,1	0	34± 2,2	1½± 1
after and op. severe insuf.	9.9	45	7	0	0	0	46½	1½
			Dog 146					
after 1st operation	9 ± 1,9	53± 0,8	7 ± 2	0	1,8± 0,5	0,4± 0,8	32± 7	6 ± 1,7
after and op. good	11.5± 1,1	51± 0,9	13 ± 4	0	3 ± 1,3	0	27± 3	6 ± 1
after and op. light insuf.	9.8± 0,4	60± 9	11 ± 6	0	2 ± 0,8	½± ½	20± 1	6 ± 2
after and op. severe insuf.	18.4	49½	26	0	3½	½	16	5

SUMMARY

Observations on 5 adrenalectomized dogs, which were examined several times during a period of months after the operation, showed that there were no important changes in either the red or white blood cell picture before and after adrenalectomy. The changes in clinical behavior are much more important than the non-significant differences between the data of the blood picture of normal and insufficient dogs. An increase in hematocrit value during severe insufficiency could be demonstrated only in 2 dogs.

We sincerely thank Organon Ltd., Oss, Holland, for the supply of cortin required.

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CLINICAL AND CHEMICAL OBSERVATIONS ON ADRENALECTOMIZED DOGS MAINTAINED BY A DIET HIGH IN SODIUM SALTS AND LOW IN POTASSIUM[1]

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THE PROLONGATION of life of adrenalectomized dogs by the administration of sodium salts appeared, until 1935, to be but a palliative measure in warding off death for a few days (1). In that year experiments were reported by Harrop, Soffer, Nicholson and Strauss (2) and Allers (3), in which the sodium and chloride intake were so regulated that the maintenance of life for relatively indefinite periods without the use of cortical hormone was accomplished. Animals thus treated are normal in external appearance and behavior in the regulated life of the laboratory. This dietary therapy cannot be termed complete substitution therapy, however, for the health of these dogs may be endangered by slight change in the diet or environment. Nevertheless, such studies have thrown a considerable light on the function of the adrenal cortex and provided a new physiological preparation for various types of study.

An important feature in the successful maintenance of dogs on a high Na intake is recognition of the toxic action of potassium, first emphasized by Hastings and Compere (4) and so well brought out by the work of Zwemer and Truszkowski (5, 6). However, to Kendall and his associates (7) belongs the credit for the development of a diet low in K but high in Na salts which is taken readily by most adrenalectomized dogs and which, without cortin, sustains life for months.

Our desire to maintain adrenalectomized dogs by the above means was not primarily for the conduct of electrolyte studies but for the investigation of sympathetic nerve function in the absence of cortical hormone reported previously by Cleghorn, Armstrong and Austen (8). In our endeavors to maintain adrenalectomized dogs on the high salt, low K diet, we encountered some difficulties not emphasized by Kendall or his colleagues. It was felt that an account of these experiences and of certain other clinical and chemical observations made during the course of the work might be of benefit to others interested in this field.

METHODS

Healthy male dogs were adrenalectomized in 2 stages by the lumbar approach or at one operation through a right rectus incision. They were treated with adrenal

[1] Read before the Association for the Study of Internal Secretions, St. Louis, Missouri, May 12, 1939.

cortical extract for some days or weeks after operation, and for a day or two after being put on the high salt, low K diet. This diet was at first made up of the materials listed by Allers and Kendall (9). Our initial diet contained rather larger amounts of sucrose than used later, and rather less salt. Most of our fatalities occurred in animals on this regimen. With the appearance of Nilson's paper (10), his more detailed diet was adopted in principle. As made up by us it contained: casein 12.5%, cracker meal 15%, sucrose 10%, lard 10%, butter (salt free) 5%, yeast 2%, cod-liver oil 1%, salt mixture (iron, magnesium, etc.) 0.5%, NaCl 2.5%, sodium citrate 1.5% and water 40%. The animals received 200 gm. of this mixture twice daily. Variation in the diet was supplied by substituting portions of twice extracted, cooked meat, bread or gravy. Refusal of food was treated by force feeding. In place of water, 0.5 to 0.7% saline was supplied. The room temperature was thermostatically regulated to 72°F. Methods used for chemical analyses were: Na, Butler and Tuthill; K, Shohl and Bennett; N.P.N., Folin Denis; sugar, Hagedorn-Jensen.

The crisis in salt maintained dogs. A few days after starting a dog on the high NaCl, low K diet and discontinuing extract injections, the animal may fairly be considered to be existing without the influence of the cortical hormone. The artificially high NaCl intake prevents the development of the circulatory collapse which ordinarily occurs on a low salt diet. Addition of K to the diet of the salt maintained animal, infection, and other factors may, however, lead to a failure in maintenance and death. The signs of this untoward development show certain characteristic differences from the signs of adrenal insufficiency developing in an animal on an ordinary meat diet with low NaCl content after cessation of cortin injections. Under the latter conditions severe signs of insufficiency do not as a rule appear for several days after cessation of extract injections. Anorexia develops gradually and also weight loss. Diarrhea and vomiting are infrequent happenings and, in our experience, never severe, occurring terminally after weakness is well marked and the extremities obviously cold and death is but a few hours off.

In the salt maintained dogs, on the other hand, the change in the external appearance and behavior of the animal is usually sudden. One day it is bright and active and eating well. The next morning it may be found to have vomited. The vomitus may contain free blood. The passage of tarry or loose, strawberry colored stools is often observed. In spite of such changes the animal may still seem bright and active. In a few hours the eyes appear sunken, lethargy develops, the extremities become cold and the heart rate fast, later slow and irregular. Râles and rhonchi may be auscultated and the breathing becomes labored and rapid. Then if treatment is not instituted, or at times in spite of it, death occurs a few hours later in convulsions. In view of the rapid and dramatic development of these changes, we have used the term crisis, as has Nilson (10) to designate the occurrence of this state of insufficiency in the salt maintained dog. Less frequently, loss of appetite and the passage of tarry stools are the only signs of inadequate treatment. This condition may last some days until remedied by treatment or until developing into the phase of acute collapse.

RESULTS

Analysis of the data summarized in table 1 shows that in 3 and probably 4 animals where treatment failed to alleviate crises, death could have been avoided by certain measures. In Experiment 1, the presence of worms no doubt contributed largely to the crisis, a fact observed by Allers and Kendall (9). Our routine anthelmintic treatment had apparently been inadequate in this animal and the presence of the parasites was not suspected.

The death of the animal in Experiment 2 was the result of inexperience, it being the first tried on the diet. Though it appeared lethargic, vomited and had a loose stool during the day, the only treatment was force feeding and 2 intravenous injections of 200 cc. of normal saline and glucose in the evening. This was inadequate and death occurred 7 hours later during the night. We had failed to recognize the gravity of the symptoms or attach serious importance to the sunken appearance of the eyes. The crisis in the dog in Experiment 4 was probably due to the gross omission to vaccinate against distemper. The failure of intensive treatment in Experiments 3 and 8 is

TABLE 1. SUMMARY OF RESULTS OF MAINTENANCE OF ADRENALECTOMIZED DOGS ON HIGH SODIUM CHLORIDE, LOW POTASSIUM DIET

Exp. no.	Date of adrenalectomy	Days treated with cortin[1]	Days on salt diet	Remarks
1	1/18/37	11	36	Crisis on 34th day. Died in convulsions 36th day in spite of repeated intravenous injections and force feeding. Autopsy showed ileum packed with worms.
2	11/10/36	63	39	Crisis on 38th day inadequately treated. First dog tried on diet.
3	1/4/37	43	41	Crisis on 40th day. Repeated intravenous injections of normal saline and glucose failed to restore. Used for blood pressure experiment.
4	1/24/37	10	45	Ate poorly last 14 days on diet. No improvement with repeated intravenous saline and forced feeding. Nasal discharge, probably distemper. Used for blood pressure experiment 45th day.
5	8/30/37	21	53	Spontaneous hypoglycemia on 53rd day after 18 hours' fast associated with eczema. Restored by cortin.
6	2/11/37	16	55	Crisis purposely precipitated by K administration. Restored by cortin.
7	1/4/37	11	109	Used for blood pressure experiment 109th day. Crises on 56th and 98th days successfully treated without cortin.
8	8/30/37	14	115	Crisis on 114th day. Intensive treatment failed to restore.
9	7/12/37	63	127	Used for blood pressure experiment on 127th day. Crises on 38th, 101st and 116th days successfully treated without cortin.
10	10/19/36	86	146	Used for blood pressure experiment 146th day in early crisis. Crisis 77th day successfully treated without cortin.

[1] Cortin not used in treatment of crises except as indicated. Refers to period immediately following adrenalectomy and before starting salt diet. This includes days off extract for testing.

more difficult to understand for success attended similar treatment utilized in the more severe crisis shown by the dog in Experiment 7, to be detailed later. It is probably significant that in Experiment 3 no urinary flow was established, and in Experiment 8 the excretion was but slight after the institution of therapeutic measures. In Experiment 5, hypoglycemia occurred spontaneously though in association with the development of eczema. The blood sugar was found to be 10 mg.% and cortin as well as saline was used. Recovery took several days. The crisis in Experiment 6 was purposely induced by K, and cortin used to restore the animal which was later used again.

The aspect of salt maintained, adrenalectomized dogs when eating well is that of a normal animal. They play and fight if opportunity arises and, as Nilson (10) has shown, are in a positive balance in so far as NaCl and water

are concerned. We have not seen hypoglycemia occurring as a result of these exertions, such as described by Harrop and his coworkers (2).

Course and treatment of salt maintained dogs in crisis. In order to gain a clearer idea of the changes occurring in the crises of salt maintained dogs it is necessary to examine the details of certain of the animals. Experiment 3 is particularly instructive, and the course and treatment during a crisis and the blood findings are described below and in figure 1.

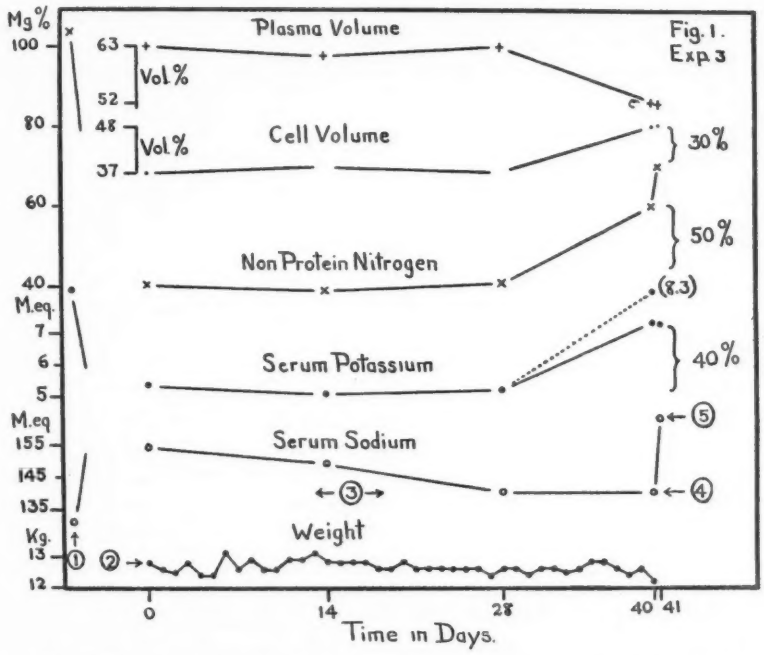

Fig. 1. Blood values and body weight of an adrenalectomized dog (Exp. 3): (1) in adrenal insufficiency developing when on an ordinary meat diet, 9 days after cessation of cortin injections; (2) hiatus represents 18 days of cortin treatment; (3) during maintenance on high NaCl, low K diet; (4) in crisis; (5) on verge of death after 19 hours' treatment. Point at end of dotted line represents the calculated value for serum potassium as expected from water loss. M. eq. in this and other figures indicates milli-equivalents. Plasma volume means relative plasma volume (from hematocrit).

Experiment 3. The animal appeared bright and active and ate well up to and including the 39th day on the high salt diet. At 9:00 A.M. on the 40th day (March 29, 1937) the dog appeared lethargic, its eyes sunken, and had a loose stool and vomited. The heart rate was 165 per minute (normal for this dog about 95). Results of blood analysis were Na, 139.5 m. eq.; K, 7.2 m. eq.; N.P.N., 59 mg.%; hematocrit 48% (normal 37 %); sugar 92 mg.%. At 10:00 A.M. 200 cc. normal saline and glucose was given intravenously. About 50 gm. of the diet was force fed. At 12:01 P.M. the animal was force fed as before. At 3:30 P.M. 300 cc. normal saline was given intravenously. At 5:00 P.M. the animal vomited. At 5:30 P.M. 230 cc. normal saline was given intravenously. The animal was still very lethargic and at 8:00 P.M. loose stool was observed. At 8:30 P.M. intravenous 250 cc. normal saline was given. Heart rate was 140. The animal was force fed. At 10:30 P.M. the extremities were cold, heart rate was 118, rectal temperature was 38°C. At 12:01 A.M. vomiting occurred. The food was force fed. Eyes became more sunken. At 12:05 A.M. intravenous 200 cc. normal saline, glucose

and 0.35 gm. sodium bicarbonate were given. The animal breathed wheezily. Coarse râles were auscultated in the chest. At 1:00 A.M. the dog was shivering, shaky and coughed wheezily. At 2:45 A.M. the eyes were still more sunken, the heart action irregular. At 3:00 A.M. the heart rate was 130. Intravenous 150 cc. 4% saline with 0.35 gm. of sodium bicarbonate was given. The dog vomited clear fluid immediately afterwards, the heart was failing, rate 72. At 4:40 A.M. loose, strawberry colored stool of about 200 cc. was observed. The heart rate was 69, rectal temperature 38.7°C. At 5:10 A.M. the dog was prostrate. Blood analysis gave the following results; Na, 162 m. eq.; K, 7.2 m. eq.; N.P.N., 69 mg.%; hematocrit 50%; sugar 70 mg.%. Eighty mg. nembutal was injected intravenously. The carotid blood pressure was 100 mm. Hg. Autopsy was performed after blood pressure experiment. Stomach and small intestine were empty and the mucosa congested. No ulcers or worms were found. The mucosa of the colon was intensely congested and the lumen contained considerable pink fluid material. The lungs were very edematous. Adrenal sites were clear.

In figure 1, the blood chemical values in the period of adrenal insufficiency prior to the starting of the high salt, low K diet regimen should be noted. Unfortunately a red cell volume was not obtained then, but the serum electrolyte and N.P.N. values show much more striking departure from normal than at the time of crisis after 40 days on the high salt diet. The absolute level of these constituents in the blood does not seem, therefore, to be of paramount importance. The precipitation of the crisis in this dog on the 40th day cannot be assigned to any definite factor. Elimination of K may have been inadequate and slow accumulation of this toxic constituent may have led to the blood changes illustrated in figure 1. Possibly the serum sodium value of 140 m. eq. on the 28th day should have served as a warning. The fact that the value found for Na at the time of the crisis was not lower than that found on the 28th day does not mean that Na was not lost from the blood stream. It only signifies that the fluid lost from the blood stream contained the same concentration of Na as the plasma. The hemoconcentration must be borne in mind in respect to the serum K increase also. This is more apparent than real and can be more than accounted for by the plasma water loss. If a calculation of the serum K increase is made on the basis of water loss alone, the value on the 40th day would be 8.3 m. eq., so actually there seems to have been K loss. Nevertheless, there may well be a real rise in tissue and blood cell K, as Zwemer and Truszkowski (6) and Nilson (10) have shown.

Over a 17-hour period, this animal received 1330 cc. of fluid containing about 10 gm. of NaCl. Despite this intravenous fluid, the blood was more concentrated 2 hours after the last injection, though the serum sodium rose to 162 m. eq. Water must have passed out of the blood stream. Into what tissues it passed, apart from the colon and lungs, we do not know. Failure of the heart, as indicated by the slowing rate, heralded death, yet after prostration had occurred, the blood pressure was still 100 mm. Hg. In our experience, prostration does not occur in ordinary adrenal insufficiency until the blood pressure reaches 50 mm. Hg or less. At no time during the last 24 hours of this animal's life was it observed to pass urine.

In the next example to be cited, Experiment 7, the crisis occurring in the 56th day of high salt diet treatment was strikingly similar to that in Experi-

ment 3, just cited. The animal, however, responded to treatment though the severity of the crisis is attested to by the signs which were observed.

Experiment 7. Blood values were normal on the 44th day of high salt dietary period, hematocrit being 37%. On the 56th day the dog was found to be lethargic, to have lost 800 gm. in weight overnight (stool and vomitus). The blood values were Na, 134 m. eq.; Cl, 104 m. eq.; K, 6.9 m. eq.; N.P.N. 44 mg.%; hematocrit 42%. Three intravenous injections of 200 cc. of normal saline, during the day, did not significantly alter the elevated heart rate (150 per minute) nor apparently improve the animal which vomited force fed food. The dog passed urine on 3 occasions, however. On the 57th day, 12:15 A.M., 200 cc. saline with 20 gm. glucose and 0.35 gm. sodium bicarbonate were given. At 2:00 A.M. the heart rate was 110. The dog coughed wheezily, passed pale urine. At 3:30 A.M. intravenous 150 cc. of 4% saline and 0.4 gm. of sodium bicarbonate was given. The dog drank 200 cc. saline immediately, and then vomited it with 50 to 75 cc. free blood, then drank more saline. An hour later pale urine was passed; the dog seemed brighter. The heart action was very irregular, rate 100. At 10:00 A.M. 200 cc. normal saline and glucose was given. At 7:00 P.M. the dog was force fed, and was wheezy. At 10:15 P.M. the eyes were still sunken; the dog still wheezed. The dog ate voluntarily, urinated. At 11:50 P.M. 200 cc. normal saline was injected intravenously. On the 58th day at 12:10 A.M. the dog was force fed; at 10:30 A.M. blood values were Na, 152 m. eq.; Cl, 108 m. eq.; K, 5.3 m. eq.; N.P.N., 36 mg.%; hematocrit 35%.

What determined the flow of urine in this animal is not known. In some dogs dying in crisis the kidney is extraordinarily congested. This might be so severe as to prevent the organ functioning or resuming function. Possibly the most significant difference between the crisis in this animal and the one in Experiment 3 lies in the fact that the hematocrit change in Experiment 7 was only 5% while that in Experiment 3 was 11%. This supposition is weakened by evidence supplied by Experiment 10 which indicates that a crisis may occur in spite of no change in hematocrit. In these experiments intravenous injection of normal saline was not strikingly beneficial. Since we used sodium bicarbonate only in homeopathic doses nothing can be said regarding its importance. Benefit in Experiment 7 seemed to follow the hypertonic saline. Subsequent experiments bore this out.

In Experiment 10, the animal experienced a crisis on the 78th day which is of interest because only the blood Na and Cl showed changes. These are depicted in figure 2 which also shows the much more severe variation in blood chemical values in a period of ordinary adrenal insufficiency prior to starting the high salt diet.

Experiment 10. The dog ate and behaved normally up to and including the 76th day on the high salt diet. On the morning of the 77th day it was found to have vomited food, but it ate this again when offered. Blood values were Na, 153 m. eq.; Cl, 114 m eq.; K, 5.5 m. eq.; N.P.N., 38 mg.%; hematocrit 38% (value on 68th day, 37%). Since the dog appeared bright and active, ate and drank well, no intravenous injection was given. Next morning (78th day) at 9:00 A.M. there was vomitus on cage floor again; at 9:50 A.M. a loose, strawberry colored stool was passed, about 150 cc. in amount. At 10:00 A.M. blood values were Na, 137 m. eq.; Cl, 106 m. eq.; K, 5.3 m. eq.; N.P.N., 42 mg.%; hematocrit 36%. The weight was 700 gm. less than the previous day (maximal normal variation 400 gm.). At 10:00 A.M. 200 cc. intravenous 4% saline was given. At 2:15 P.M. 400 cc. saline drinking fluid was taken. Intravenous 200 cc. 4% saline was given. At 5:00 P.M. the dog looked lethargic and toxic. Heart rate, previously about 100, was now 66. Extremities were cold. At 5:30 P.M. the heart rate was 100 and irregular. Intravenous 200 cc. normal saline and 40 gm. glucose was given. The heart rate rose immediately to 180. Rate 12 minutes later was 130

The dog passed 300 cc. urine. At 10:00 P.M. the dog had taken 400 cc. saline per os and excreted 500 cc. urine. One hundred gm. diet was force fed. On the 78th day the dog ate voluntarily, on the 83rd day the hematocrit was 33%.

Although the red cell volume was not increased at the time of crisis over the values in preceding days, the fact that treatment caused a drop indicates that it had not been at an optimal value at least. The sudden decrease in the Na and Cl level in the blood from the 77th to 78th day is probably a result

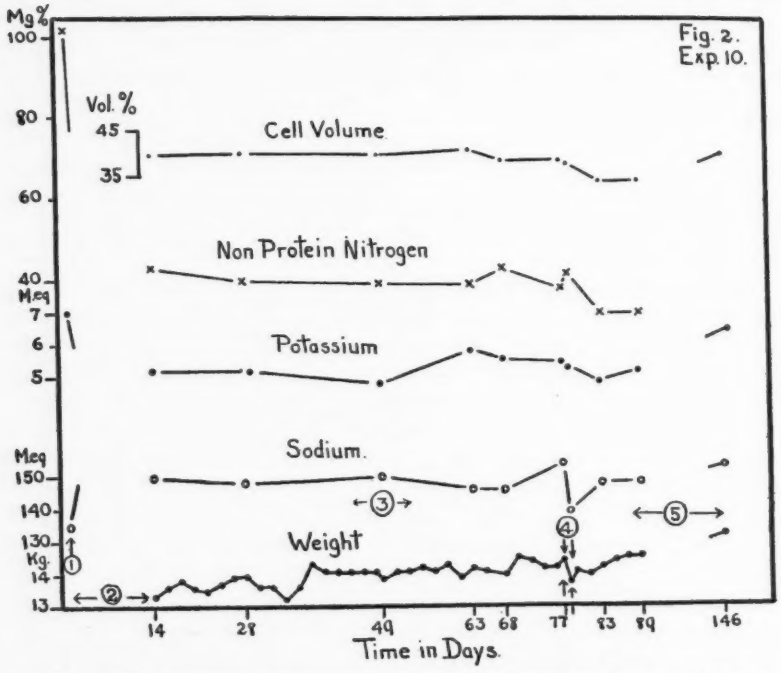

Fig. 2. Blood values and body weight of an adrenalectomized dog (Exp. 10): (1) in adrenal insufficiency developing 9 days after cessation of cortin injections; (2) hiatus represents 14 days of cortin treatment and 13 days on high salt diet; (3) during maintenance on high NaCl, low K diet; (4) in crisis; (5) after recovery and until crisis on 146th day terminated by blood pressure experiment.

of migration of salt and water into the intestine. What exactly determined this change we do not know. Most likely it is associated with the congestion of the mucosal vessels of the colon, as seen at autopsy on such animals. The initial injection of hypertonic saline was dramatically beneficial, the second very much less so. The cardiac acceleration following the intravenous glucose at 5:30 P.M. is of interest. The failure of the heart that seemed to be occurring previously may possibly have been dependent on deficient supply of carbohydrate for the heart, since Britton, Silvette and Kline (11) have shown that cardiac glycogen is severely reduced in adrenal insufficiency. Supplying glucose by vein may have repaired the accelerating mechanism.

Precipitation of crisis by potassium. No matter whether potassium is elevated in the blood serum in crisis or not, the administration of K as Allers

and Kendall (9) and Nilson (10) have shown, will precipitate a crisis. This is well borne out in Experiment 6 and illustrated in figure 3.

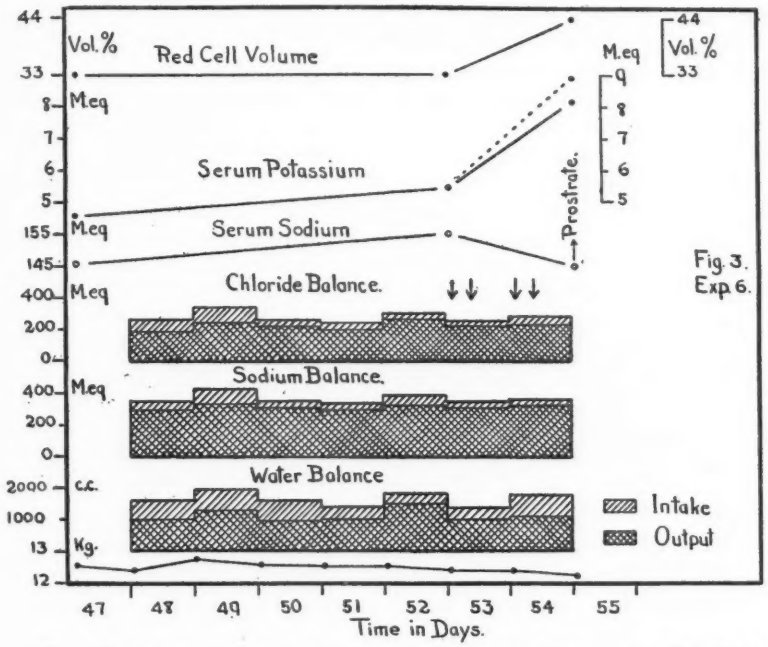

Fig. 3. BLOOD AND URINE VALUES AND BODY WEIGHT OF AN ADRENALECTOMIZED DOG (Exp. 6) during THE 47TH TO 55TH DAY OF MAINTENANCE ON HIGH NaCl, LOW K DIET. On 53rd and 54th days animal received 2 doses of 0.87 gm. of K (as the citrate salt), indicated by arrows. Note that this had no perceptible effect on the excretion of Na, Cl or water, though blood values changed considerably. The point at the end of the dotted line represents the calculated value for serum K as expected from water loss.

Experiment 6. The total fluid intake (0.5% saline) and the urine output were measured from the 46th day of the high salt diet regimen. The dog received 200 gm. of diet twice daily. This contained by analysis; Na, 1.27%, Cl, 1.19% and K, 0.034%. The animal was bright and active and eating well on the 53rd day of the high salt diet period. Blood values at 9:00 A.M. were Na, 155 m. eq.; K, 5.6 m. eq.; N.P.N., 32 mg.%; hematocrit, 33%. On this and on the 54th day the dog was force fed 2.41 gm. potassium citrate (0.87 gm. K) at each feeding. On the 55th day at 9:00 A.M. the dog was found prostrate in the cage. Weight loss was 200 gm. Little vomitus was found on the cage pan. Eyes were markedly sunken. Heart rate was 30 per minute. Electrocardiogram was taken (see Experiment *D 15*, Hall and Cleghorn). Blood values were Na, 145 m. eq.; K, m. eq. 8.2; N.P.N., 45 mg.%; hematocrit, 44%. The dog was treated with repeated doses of cortical extract and intravenous saline with glucose 200 cc. at 12:00 P.M. and 100 cc. at 2:20 P.M. Loose, strawberry colored stool was seen at 3:00 P.M. The dog was walking about shortly afterwards.

Experiment 6 shows that in the precipitation of a crisis by K administration, an increase in the renal excretion of NaCl and water is not necessarily a significant factor, a fact already shown by Nilson (10) (*dog B*). Presumably the kidneys were excreting the maximum amount of NaCl they were capable of handling, so no increased excretion was elicited by the K which did, however, lead to marked plasma water loss and considerable changes in the

electrolyte pattern of the blood. Of these changes, the hemoconcentration would seem to be the most important. Fluid and salts lost from the blood probably passed into the intestine and into other tissues as well. The K and N.P.N. increases can be explained by the reduction in plasma volume, indeed, as in Experiment 7, the plasma water loss is of such an order that one would expect an increase in potassium from 5.6 to 8.9 m. eq.

A summary of typical blood findings in ordinarily adrenal insufficiency and the crisis of salt maintained dogs is contained in table 2. The points to which we wish to draw attention are the extreme elevation of the N.P.N. in ordinary adrenal insufficiency compared to that in the crises of the salt dogs, and the fact that the serum potassium and hematocrit are not necessarily altered in crisis and may be altered without the occurrence of crisis.

TABLE 2. BLOOD VALUES OF DOGS (*A*) NORMAL, HEALTHY; (*B*) TREATED, ADRENALECTOMIZED; (*C*) IN ADRENAL INSUFFICIENCY; (*D*) IN CRISIS ON HIGH NaCl DIET

Exp. No.	State	Serum		N.P.N.	Cell volume
		Sodium	Potassium		
		m. eq.	*m. eq.*	*mg. %*	*%*
		(A)			
Normal	Healthy	153 (±5)	5 (±0.5)	35 (±7)	variable[1]
		(B)			
14	Cortin treated	153	5.3	41	51
		(C)			
ibid.	Severe insufficiency 11 days off cortin	133	6.6	171	56
		(D)			
3	Severe crisis 40th days on salt	140	7.3	59	48 (+10)
7	Severe crisis 56th day on salt	134	7.1	44	42 (+7)
10	Moderately severe crisis 78th day	137	5.2	42	36 (No change)
9	No crisis 119th day but eats poorly and has tarry stools. Is bright and active.	135	6.4	58	36 (+3)

[1] Varies in different dogs but constant within ±5% under standard conditions.

DISCUSSION

The NaCl intake and output in salt maintained, adrenalectomized dogs is so high that it virtually amounts to a perfusion of the animals' kidneys with a saline solution. This compensates to some extent for the loss of the kidney's power to regulate excretion by washing out K, nitrogenous and other constituents. The reduction in the kidney's capacity to handle these waste products without the aid of a large amount of salt and water is probably not the only functional limitation in these animals whose power of adaptation is diminished in other respects, e.g., to temperature changes and infection. Hence signs of insufficiency are encountered not infrequently. These signs, as already pointed out, differ from those of ordinary adrenal insufficiency which develop following cessation of cortin injections in a dog eating a meat

diet of low NaCl content. They differ probably on account of the degree of hormone deficiency and on account of the state of hydration of the animal. In the former case, the dog is relatively dehydrated when the circulation finally fails. In the latter, there is not the same loss of body fluid though some may escape from the blood into the intestine. Nevertheless, the loss of plasma water is comparable in the two conditions. We suspect that it may be relatively greater in ordinary insufficiency, but adequate data on this point are lacking.

It is not possible to say what is the cause of crisis in many cases. Possibly accumulation of K over a period of time goes on until a toxic threshold is reached. Nilson (10) has shown that the concentration of K in red blood cells increases before a crisis. Probably many other cells in the body share in this process, a supposition supported by observations of Zwemer and Truszkowski (6). Speaking of potassium poisoning in normal and adrenalectomized dogs, Allers, Nilson and Kendall (7) say, "The severity of the symptoms produced appears to be a measure of the deficiency of cortin rather than a measure of the elevation of potassium in the blood." With this we cannot agree because we feel that in an adrenalectomized dog, after 50 or 30 or even 10 days' maintenance on the high salt diet without hormone injections, the deficiency of cortin is complete. When the dog goes into crisis some regulatory mechanism or mechanisms have broken down. These mechanisms obviously either can function under optimal circumstances, in the absence of the cortical hormone, or are compensated for by the artificially high NaCl intake. The administration of K, infection, even exertion may, however, overtax the metabolic regulatory processes whose range of adaptation is limited in the absence of cortical hormones. Hypoglycemia may occur, presumably due to a failure in gluconeogenesis but, in our experience, the most common mechanism to break down is that which controls the fluid exchange between blood and tissues, then fluid and even blood pass out by the bowel and to a less extent by the stomach. Probably some NaCl and water pass directly into body cells, such as muscle, as well as into those of the gastrointestinal tissues. Fluid loss accounts for the hemoconcentration so commonly seen. The facts that crisis can occur, as in Experiment 10, without this change, and that moderately severe changes in electrolyte pattern can exist without crisis, as in Experiment 9, mean that another important cause or causes contribute to the crisis. The most obvious would seem to be the development of edema of the lungs, leading to difficulty in gaseous exchange. Certainly labored breathing is frequently observed in the crisis occurring in salt maintained dogs and is associated with the presence of râles and rhonchi. These changes are seldom if ever encountered in dogs dying of ordinary adrenal insufficiency. Anoxemia of respiratory origin rather than circulatory failure may be a very important feature in causing the death of salt maintained dogs. In some of our experiments, e.g., Experiment 7, the animals gave evidence of having edema of the lungs over quite a number of hours, yet recovered. Therefore, we feel that this is usually but one of several points of breakdown in the organism. The second, quite apart from hemoconcentration, seems to be the heart. This is generally found to be accelerated in crisis

probably as a result of carotid sinus and other reflexes activated by reduction in circulating volume of fluid. The ultimate decline of the animal definitely coincides, in our experience, with a slowing of the heart rate which may eventually reach absurdly low figures, at which time definite block exists (12). Even then recovery can be effected by the intravenous injection of hypertonic saline or glucose (unpublished observations). The effect of such injections on these bradycardiac hearts is dramatic, acceleration as a rule following the injection almost immediately. The initial stage in the decline of the heart rate, at least, may be due largely to a defect in cardiac carbohydrate stores, for in Experiment 10 acceleration followed glucose intravenously.

Cardiac failure ultimately develops in adrenalectomized dogs whether dying of ordinary adrenal insufficiency or, if salt maintained, of a crisis. In both cases a considerable period of overwork occurs when the heart is accelerated to compensate for a reduced circulating volume of fluid. This reduced volume is shown by the hematocrit, but what is not shown is the actual reduction in circulating fluid. This, in the crisis of salt maintained dogs, is probably even more considerable than in ordinary adrenal insufficiency, as the congestion of the gastrointestinal tract is extraordinarily severe. What leads to the development of this shunting of the circulation, we do not know. Possibly accumulation of K or other unexcreted toxic metabolites is the cause of capillary dilatation, and consequent stasis and fluid loss. If K is the cause, it is not reflected in a real increase of this element in serum, for a calculation on the basis of water loss shows that the expected value is greater than that found. Whatever the cause, the chief defect seems to be a reversal in the direction in which fluids and salts pass from the circulation, and cells of the gastrointestinal tract.

SUMMARY

The results of efforts to maintain 10 adrenalectomized dogs alive on a high NaCl, low K diet are reported. The difference in the development and in the signs of ordinary adrenal insufficiency and the crisis of salt maintained dogs is emphasized. The clinical signs of the latter are correlated with the blood chemical and pathological findings. An experiment is reported in which K administration led to a crisis without the occurrence of a diuresis. It is pointed out that the serum K increase in this and other experiments is often less than that to be expected from the blood water loss. The interpretation of the findings is discussed.

We would like to express our appreciation to Prof. Duncan Graham for his continued interest in this work.

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THE EFFECT OF A SINGLE INJECTION OF THYROXIN ON CARBOHYDRATES, PROTEIN AND GROWTH IN THE RAT LIVER[1]

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IT HAS BEEN customary to study the effect of thyroid substances on the chemical composition of body organs by feeding thyroid or injecting thyroxin at daily intervals and then examining the organs some time after the last injection. In spite of the fact that the amount of thyroid material, the duration of administration, and the time of the chemical analysis varied considerably in these investigations, some results, as for instance the loss of glycogen, proved to be rather constant and have been considered as characteristic for the action of thyroid substances.

It seems, however, that the method of studying the effects of thyroid material after repeated administration and at an arbitrarily chosen time after the last dose is open to criticism. Thyroid action, when measured by the effect of a single dose upon the oxygen consumption of the animal, is not uniform throughout the period of its activity, but varies according to the time elapsed after the injection. There is, first, a 'latent period,' in which the oxygen consumption remains unchanged; then follows a phase of increasing metabolism; and finally a period in which the oxygen consumption falls to normal or subnormal values. With a single injection of thyroxin in the rat, these periods are 18 hours, 4 to 6 days, and 3 to 9 days, respectively, for doses varying between 2 and 4 mg. per kg. of body weight (1). It would appear that the chemical processes, furnishing the energy for the increase in metabolism, must vary correspondingly in magnitude and direction. Also, changes in the chemical composition of body organs might be different, depending on the time elapsed since the administration of the thyroid preparation. A single analysis of a body organ, therefore, is hardly representative of the entire duration of the effect. It is even more difficult to interpret the significance of a single analysis after repeated administration, because the time effect of the second, third and following doses will overlap the time curve of the first dose and disguise the course of the chemical reactions. A better insight into the development of the chemical changes within the organs can be obtained by studying the effect of a single dose over a prolonged period of time. By establishing the time curve of the chemical changes a clearer insight into their alleged relationship with the variations in oxygen consumption might be expected.

[1] Presented in part before the Chicago Society for Internal Medicine, November 28, 1938. A preliminary communication of part of the chemical results has been published previously (28).

In the present study, the effects of a single injection of thyroxin upon the rat liver have been followed by killing the animals at regular intervals. Fresh weight, dry weight, glycogen, glucose and lactic acid content of the liver, and the variations in total nitrogen, non-protein nitrogen, amino acid nitrogen and ammonia nitrogen have been determined. The relationship of these changes to the curve of oxygen consumption over a prolonged period after the injection of thyroxin will be discussed. It will be shown that chemical variations occur similar to those described in growing tissues. Finally, it will be demonstrated by histological studies that growth takes place in the liver under the conditions of these experiments.

METHODS

Male albino rats, weighing between 150 and 220 gm., were kept at a room temperature of about 23° C. They were fed a uniform diet of milk, bread and lettuce, and care was taken that food was available at all times. The animals were divided into groups; every 4th or 5th animal served as a control. Each experimental animal received a single injection of thyroxin (Schering), 4 mg. per kg. of body weight. The injection was performed at such times that each animal was killed between 8 and 9 o'clock in the morning. At the interval given, the animal was decapitated and the liver quickly excised. A sample of about 500 mg. was removed for the determination of dry weight; the remainder of the liver was wrapped immediately into a sack of dense gauze, placed in liquid air and crushed into small pieces. Samples of 50 to 120 mg. were weighed in a frozen state on a torsion balance, and analyzed separately for glycogen, glucose and total nitrogen. For each determination, triplicate samples were used. The remaining liver was powdered under liquid air and the powder was divided into 3 portions of about 1.5 to 2 gm. each. One part of the powder was used for the determination of N.P.N. and amino acid nitrogen, the second portion for lactic acid determination, the remainder for the determination of ammonia. The traces of liver powder adherent to the gauze were minimal, so that the weight of the total liver was given by addition of the weights used in all determinations together. Each liver was analyzed separately. The figures listed represent the average value of a number of these analyses. The liver weight is expressed per 100 gm. body weight of the animal at death. The chemical constituents are also expressed in terms of 100 gm. body weight, since the liver showed regularly an increase in fresh weight and dry weight after injection of thyroxin.

The glycogen determinations were performed by a micromodification of Pflueger's method, using alcohol saturated with sodium chloride (2) for the precipitation of glycogen, and the method of Hagedorn and Jensen (3) for the determination of glucose. In many instances up to 6 glycogen analyses were performed on pieces of the same liver. The average deviation observed in 500 analyses was $\pm 7.0\%$. Determinations of lower carbohydrates were performed by a micromethod described by Lohmann (4). Since in the liver this fraction consists mainly of glucose, it is simply referred to as glucose in this paper. Lactic acid was determined by Lehnartz' modification (5) of the Hirsch-Kauffmann-Embden method (6, 7). Determinations of total nitrogen and N.P.N. were carried out with a modified micro-Kjeldahl apparatus, using ground glass stoppers and glass connections throughout. The samples were digested with the Arnold-Gunning mixture, followed by the addition of hydrogen peroxide. For N.P.N. determinations the powdered liver was deproteinized with 4% trichloracetic acid; the ammonia was distilled into N/100 sulfuric acid and the titra-

tion carried out with Tashiro's indicator (8). For details see Embden (9). The amino acid determination was made as described by Folin (10), the ammonia determination as given by Embden (9). Blood sugar was determined by the Hagedorn and Jensen method in blood obtained by decapitation.

In the histological studies, rats weighing between 230 and 280 gm. were used; the dose of thyroxin was again 4 mg. per kg. of body weight. Sections of each liver were stained with hematoxylin-eosin, with Best's stain for glycogen, and with Kernechtrot for mitoses.

RESULTS

As demonstrated in table 1, the liver shows an increase in fresh weight and dry weight per 100 gm. of body weight, beginning 12 hours after the injection of thyroxin. The weight reaches a peak after 48 hours, but still remains higher than normal in the following intervals.

TABLE 1. EFFECT OF A SINGLE SUBCUTANEOUS INJECTION OF THYROXIN (4 MG. PER KG. BODY WEIGHT) ON LIVER WEIGHT, GLYCOGEN, GLUCOSE, LACTIC ACID AND NITROGEN CONSTITUENTS OF THE RAT LIVER

(Values calculated per 100 gm. of body weight)

Time after injection	No. of animals	Liver weight		Glycogen	Glucose	Lactic acid	Total N	N.P.N.	Amino acid N	Ammonia N
		wet	dry							
hr.		*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Controls	17	2.90	0.865	112.6	31.7	2.1	77.3	5.0	1.9	0.2
3	4	2.89	0.857	112.7	—	2.0	81.8	5.1	1.8	0.2
6	9	2.89	0.851	56.2	29.4	2.0	85.1	5.3	1.8	0.2
12	7	3.23	0.938	77.3	34.8	2.4	90.0	5.4	2.2	0.2
18	3	3.30	0.965	55.1	29.9	2.1	94.2	5.0	2.2	0.2
24	11	3.31	0.960	54.9	28.1	2.9	93.3	5.6	2.0	0.3
36	5	3.28	0.923	61.6	42.6	2.1	95.8	6.3	2.5	0.2
48	9	4.11	1.104	46.0	33.1	2.4	123.7	6.7	2.8	0.3
60	7	3.19	0.906	81.4	51.5	2.3	97.0	5.7	2.1	0.3
72	4	3.46	0.906	136.9	67.6	3.0	82.3	4.8	2.5	0.3
84	2	3.24	0.954	168.8	54.1	1.8	81.7	6.5	1.7	0.3
120	1	3.68	1.053	158.2	24.2	2.2	97.3	6.3	2.7	0.2
144	1	3.63	1.136	142.2	53.5	2.9	111.2	5.8	2.1	0.2

The chief loss in glycogen occurs between the 3rd and 6th hour after injection. The glycogen content remains low up to the 48th hour, rises markedly until the 84th hour to values above normal, then begins to decrease. The decrease in glycogen, which occurs early in the 'latent period,' is not accompanied by a rise in liver sugar. There occur only moderate variations of liver sugar between the 24th and 48th hour. Then, however, the liver sugar rises, simultaneously with the increase in glycogen, to more than twice its original value at the 72nd hour, after which time it decreases. No significant changes occur in the lactic acid concentration.

Loss and increase of glycogen are also observed in determinations of the glycogen content of the whole animal (table 2), demonstrating that the variations in liver glycogen cannot be explained by a shift of glycogen from the liver to other organs or vice-versa.

Table 3 presents the variations of the blood sugar, determined simultaneously with the liver glycogen in a series of litter-mate male rats. There is no change in blood sugar during the first 9 hours, when the loss in liver

TABLE 2. EFFECT OF A SINGLE SUBCUTANEOUS INJECTION OF THYROXIN (4 MG. PER KG. BODY WEIGHT) ON THE GLYCOGEN CONTENT OF THE WHOLE ANIMAL

(Values calculated per 100 gm. of body weight)

Time after injection	No. of animals	Glycogen
hr.		*mg.*
Controls	10	205
24	8	160
48	9	174
60	4	234
72	4	279
96	2	315

glycogen is observed. On the other hand, there occurs a marked increase in blood sugar at the 72nd hour, when the liver glycogen shows its rise. In this instance, the glycogen content at the 72nd hour is slightly below the control value; the peak of the liver glycogen curve, which might be expected at the 84th hour, was not determined, since no more animals of this litter were available. At the 96th hour, the blood sugar has returned to normal, showing a close parallelism to the variations in liver sugar in table 1.

TABLE 3. EFFECT OF A SINGLE SUBCUTANEOUS INJECTION OF THYROXIN (4 MG. PER KG. BODY WEIGHT) ON LIVER GLYCOGEN AND BLOOD SUGAR IN A SERIES OF LITTER-MATE MALE RATS

(Values calculated per 100 gm. of body weight)

Time after injection	Liver weight		Glycogen	Blood sugar
	wet	dry		
hr.	*gm.*	*gm.*	*mg.*	*mg. %*
Control	3.17	1.005	158.2	109
3	3.44	1.087	154.0	109
6	3.81	1.154	75.4	110
9	3.58	1.124	50.0	117
9	3.29	1.187	54.2	121
48	3.50	1.130	60.0	116
72	3.68	1.233	140.3	160
96	3.82	1.203	147.9	112

The total nitrogen content of the liver increases steadily to the 48th hour, drops off by the 84th hour to a value still above normal, and increases again to the 144th hour. In contrast to these changes in total nitrogen, those of the N.P.N., amino acid nitrogen, and ammonia nitrogen are insignificant. The rise in nitrogen, therefore, must be due to an increase in protein.

A comparison of the protein values, obtained by multiplying the difference between total nitrogen and N.P.N. by 6.25, with the values of total carbohyrates in the same liver reveals an interesting reciprocity: carbohydrates decrease as proteins increase, and vice versa (fig. 5). The percentages given are for dry weight and expressed in percentage of normal liver (liver weight per 100 gm. body weight). The inverse relationship between protein and carbohydrates is most marked between the 48th and 84th hour, and becomes almost linear, if the amount of convertible protein (59% of the total protein) is compared with the corresponding carbohydrate values.

The study of the chemical variations was supplemented by histological examination at the same intervals after a single injection of thyroxin as for the chemical analyses. As pointed out before, the liver increased in size. This enlargement was not only a rise in water content but also increase in dry weight. Was the increase in dry weight simply an expression of the alternating rise of protein and carbohydrates, or was it due to an active proliferation of liver tissue? This question could be answered only by histological examination.

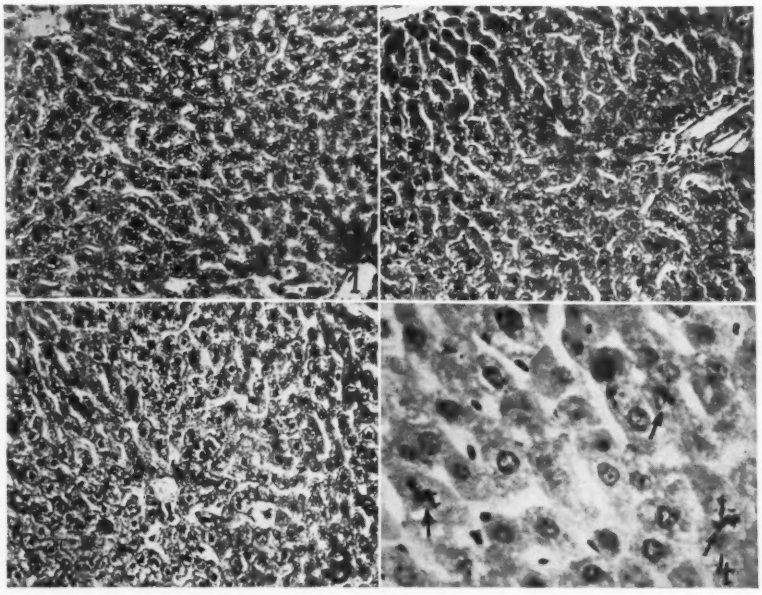

Fig. 1. NORMAL RAT LIVER. Hematoxylin-eosin stain. ×112.

Fig. 2. LIVER OF A RAT KILLED 72 HOURS AFTER INJECTION OF THYROXIN (4 mg. per kg. body weight). Hematoxylin-eosin stain. ×112. The cells toward the periphery of the hepatic lobule appear hazy; the delineation between the cells is less distinct than in the normal liver. The cell nuclei vary in size.

Fig. 3. LIVER OF A RAT KILLED 96 HOURS AFTER INJECTION OF THYROXIN. Same dose, stain and magnification as in figure 2. The liver cells are increased in size; there are many binuclear cells and 'large white cells.' Irregular formation of the cells; the trabeculae cannot be outlined.

Fig. 4. MITOSES IN THE LIVER OF A RAT KILLED 72 HOURS AFTER INJECTION OF THYROXIN. Kernechtrot stain. ×338. Mitoses indicated by arrows.

Sections of livers, taken at regular intervals after the injections of thyroxin and stained with hematoxylin-eosin, show marked changes in the cell structure. Beginning 12 hours after the injection and increasingly pronounced up to the 72nd hour, the cells toward the periphery of the hepatic lobule lose their regular polyhedral form, are less clearly stained and appear hazy. Consequently, the delineation between the cells becomes less distinct, and toward the 96th hour the aspect of the peripheral part of the lobule is quite irregular, so that a distinction of the trabeculae cannot be made (fig. 1, 2, 3). The nuclei of these cells become less visible and vary in size, in contrast to

the uniformity of the nuclear size in the cells of the normal liver. At the 96th hour, an increase in the number of binuclear cells is observed. Many of these cells have a very light-colored protoplasm, are markedly enlarged and correspond to the 'large white cells' described in regenerating liver (11).

The glycogen content of the liver cells, as indicated by Best's stain, varies consistently with the morphological changes. Twelve hours after injection, no glycogen can be detected with this stain. With Carnoy's fixation reagent, however, a scanty distribution of glycogen in the form of discrete fine dots can be observed. The amount and size of the glycogen particles is still reduced at 24, 36 and 48 hours; after this time there occurs a marked increase in glycogen. At the 84th hour, in some slides at the 108th hour, corresponding to the period of the increase in cell size, the liver cells contain large amounts of glycogen, so that they seem distended by huge glycogen particles. Later on the glycogen content decreases.

Finally, in a series of stains with Kernechtrot, special attention was directed to the occurrence of mitoses. While no mitoses are seen in the liver of normal rats of this size, in the thyroxin-injected rats an occasional mitosis occurs even 12 hours after injection. In the 24-hour specimens there appear more mitoses, and in the 48, 60 and 72-hour specimens, numerous cells in different phases of mitosis can be observed. Figure 4 shows a typical specimen of a liver, 72 hours after injection, with 4 mitoses in one high power field. The similarity of the changes in cell structure to those described in regenerating liver, following experimental ablation of parts of the liver (12), and the great number of mitoses, seem to demonstrate beyond doubt that an active cell proliferation takes place in the liver as an effect of a single injection of thyroxin.

DISCUSSION

The results of these experiments indicate that the changes in the chemical composition and histological structure of the rat liver after a single injection of thyroxin are a function of the time elapsed after the injection. It is particularly significant that the first effects are noted early in the latent period, before the oxygen consumption begins to increase.

The chief loss of glycogen occurs within 6 hours after the injection; it precedes, therefore, the rise in oxygen consumption, which begins about 12 hours later (1). Hence, it is not possible to attribute the decrease in liver glycogen to the calorigenic action of thyroxin (13). Cramer (14) assumed that the diminution of glycogen was due to an accelerated formation of liver glucose and its immediate combustion by the increase in oxygen consumption. As demonstrated in table 1 and 3, however, liver sugar, blood sugar and lactic acid content remain unchanged, when the decrease in glycogen is found. There is, therefore, no indication for an increased sugar formation during this time; nor is there a basis for the assumption that the newly formed sugar would be burned immediately by the increased oxygen consumption. The rise in metabolism occurs much later. That there is no inverse relationship between glycogen content and oxygen consumption is shown by the rise of the glycogen values, in the liver and in the entire

animal, between the 48th and 84th hour, when, in experiments under identical conditions (1), the oxygen consumption is rising towards its peak. As another explanation, it has been suggested (13) that thyroxin might exert a specific action upon the liver cells, decreasing their power to make and store glycogen. This assumption cannot be accepted without reservations, because it would be necessary to limit this action to the first 48 hours; the subsequent rise of glycogen and oxygen consumption would then have to be considered as secondary effects. It is difficult to reconcile this theory with the fact that the injected thyroxin is not entirely excreted within 48 hours (15).

In view of the discussion as to whether thyroxin causes protein breakdown in the organs or a mobilization of reserve protein (16), it is significant that the exeriments demonstrate an increase in liver protein throughout the period observed. If, therefore, a single injection of thyroxin causes increased cellular breakdown or a mobilization of reserve protein in the rat, this must occur in organs other than the liver. Increase in total nitrogen (17) and protein content of the liver after repeated injections of thyroxin have been reported even when the animals were fasted (18); and the question has been raised whether this increase in protein could be connected with a work hypertrophy of the liver, elicited by the increase in metabolism. As demonstrated in figure 5, the increase in protein begins 6 to 12 hours after the injection, therefore, it precedes the rise in metabolism.

It is not possible to determine the source of the increase in liver protein in the first 48 hours after injection of thyroxin without a simultaneous analysis of the protein content of the rest of the body, in experiments with a controlled nitrogen balance. The reciprocity between the variations in protein and carbohydrates, as shown in figure 5, might indicate a mutual interconversion of carbohydrates and protein. As demonstrated in experiments on the artificially perfused liver (19) and on liver slices (20), the liver can transform pyruvic acid into amino acid; this reaction is physiologically reversible (21) and is oriented normally towards the formation of amino acids (22). Conversely, the ability of the liver to transform protein into carbohydrates is a well established fact. An analysis of the data during the first 48 hours after injection reveals, however, that the increase in protein is larger than the disappearance of carbohydrates could account for. On the other hand, between the 48th and 84th hour, when a transformation of protein into carbohydrates might take place, the amount of convertible protein (59% of the total protein) diminishes in almost linear proportion to the increase in carbohydrates. In view of this reciprocity in the second phase, one might suggest that the increase in liver glycogen, liver glucose and blood sugar is due to gluconeogenesis from protein. Such a transformation would necessitate additional oxygen consumption and would also account for part of the caloric effect of thyroxin action. Further evidence is necessary to support this tentative hypothesis of the interconversion of proteins and carbohydrates.

The analysis of the variations in liver weight leads to a new aspect of the possible significance of the variations in the chemical composition of the

liver. The weight of the liver (fresh and dry weight) begins to increase within 12 hours (table 1) or earlier (table 3), during the 'latent period.' Since, therefore, the increase in metabolism cannot be the cause of this enlargement of the liver, it is possible that active growth of the liver tissue takes place. A consideration of this question reveals an interesting analogy between the observed variations of the liver glycogen after the injection of thyroxin and the fluctuations of glycogen described for growing tissues.

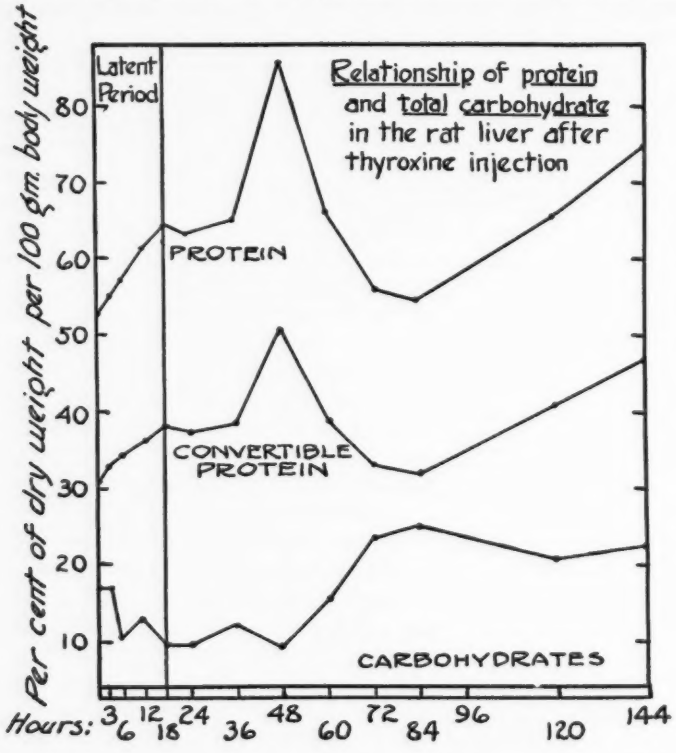
Per cent of dry weight per 100 gm. body weight
Latent Period
Relationship of protein and total carbohydrate in the rat liver after thyroxine injection
PROTEIN
CONVERTIBLE PROTEIN
CARBOHYDRATES
Hours: 3 6 12 18 24 36 48 60 72 84 96 120 144
10 20 30 40 50 60 70 80

Fig. 5

Thus, in studies on the occurrence and variations of glycogen in human embryos, Sundberg (23) observed that cells normally containing large amounts of glycogen lost this glycogen as soon as they underwent mitosis. This was true, for instance, for cells of the epidermis, parathyroids and cartilage. Jordan (24) confirmed these experiences and stated that glycogen disappeared during the active proliferation of cells, but reappeared when the newly formed cells increased in size. Similar observations were made by Harris (25) in histological studies of bone growth in a human embryo. He found a complete absence of glycogen in the zone of actual bone formation while the cells in the neighborhood of the epiphysis stored glycogen in

direct proportion to their age. The relationship between glycogen loss and cell proliferation on the one hand, and glycogen reappearance and retardation of growth on the other hand, has recently been shown convincingly by Doljanski (26) in cultures of liver cells. He succeeded in culturing liver cells in vitro to a degree of generous proliferation. These cells, however, proved to be free of glycogen. Assuming that the lack of glycogen was directly related to the rapid proliferation of the liver cells, Doljanski placed rapidly growing glycogen-free liver cells in a medium which slowed the rate of growth. Immediately, the glycogen appeared in the cells in increasing quantities.

It seems likely, therefore, that the early decrease and later increase in liver glycogen and the increase in liver size, after injection of thyroxin, must be interrelated; both could be an expression of active growth in the liver. The histological study of the liver furnishes evidence that thyroxin induces active growth. Why the cells should lose glycogen during or before mitosis is not quite understood. It is interesting that Sundberg (23) assumes that the carbohydrates are used in the formation of nucleinic acid for the nuclei of the newly formed cells. The fact that the protein content of the liver rises during this period may speak in favor of this assumption.

The evidence that thyroxin induces proliferation of the liver cells offers an interesting outlook for the explanation of the effect of thyroxin upon oxygen consumption. As a matter of fact, something similar was postulated previously by Rabinowitch (27), in a mathematical interpretation of the period of increasing heat production after thyroxin injection. He suggested that the curve of rising oxygen consumption could best be explained by the assumption that more protoplasmic mass becomes active during the rise of oxygen consumption than under basal conditions, and assumed that some kind of reserve protoplasm might be drawn into the increasing metabolic processes. It is apparent, now, that in the liver the increase of protoplasmic mass is due to growth. In view of the results with repeated injections, it is reasonable to assume that growth also occurs in other organs under the conditions of the experiments presented, thus accounting for the geometrical progression in the increment of the oxygen consumption. If this proves to be correct a satisfactory explanation could be suggested (*a*) for the latent period—as the time required for cell division and the initiation of growth; (*b*) for the rise in oxygen consumption—as due to an increase in the number of cells and the higher oxygen needs of growing cells and (*c*) for the long duration of thyroxin action. Whether growth is the only factor involved in the rise in oxygen consumption, or in addition stimulation of metabolic processes on another basis occurs, can be decided only by a quantitative study of growth in all organs in relation to the quantitative effect upon oxygen consumption.

SUMMARY

The changes in the chemical composition and histological structure of the rat liver vary according to the time elapsed after the injection of thyroxin. The chemical changes consist in a loss of glycogen in the liver and the entire

animal, followed, beginning 48 hours after injection, by an increase above normal of these values; in a marked rise in liver protein in the first period and, later, in a decrease of liver protein to values still above normal in linear proportion to the increase in liver glycogen. Liver sugar, blood sugar and lactic acid content of the liver remain unchanged during the first period, but rise later, simultaneously with the glycogen. Fresh weight and dry weight of the liver increase. The loss in liver glycogen, the increase in liver protein and in liver size begin before the oxygen consumption rises, that is, during the latent period.

The variations in carbohydrates observed in the liver present a close analogy to changes described in growing tissues. In a histological examination, changes in size and structure of the liver cells similar to those observed in regenerating liver and mitoses in steadily increasing number have been demonstrated. It is concluded that liver growth takes place under the conditions of these experiments.

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THE INFLUENCE OF REPEATED FASTING ON THE PARATHYROIDECTOMIZED ALBINO RAT

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TETANY or depression occurs in most laboratory animals following complete removal of the parathyroid glands. Hammett (1) claimed age, weight and season to be factors influencing mortality in the parathyroidectomized albino rat. Hoskins (2) confirmed Hammett's findings in regard to age and weight but did not consider season to be a factor when animals were chosen of uniform weight and age. Barnes and Fogelberg (3) found such a marked variation in mortality in animals of a given age as to require a large number to justify conclusions.

The effects of temperature (4) and fasting (5) during the first 24 hours after operation has been studied by Templeton and coworkers. When the temperature was raised the mortality increased. When the animals were fasted for 24 hours after operation the mortality was much higher than when food was given ad libitum immediately after parathyroidectomy. In view of these facts it was thought worth while to test the susceptibility of animals in different age groups to tetany in the first 24 hours and also the effects of 24 hours of fasting at weekly intervals.

EXPERIMENTAL METHODS

The animals were from the standard stock colony maintained by Loyola University Medical School. The diet consisted of Fox Chow ad libitum; bread, meat and cabbage once a week. At the ages of 30, 60, 120 and 180 days respectively 26, 45, 21 and 15 animals were parathyroidectomized. The animals were returned to their cages as soon as the operation was completed. Ingestion of food and water took place as soon as the effects of the anesthetic had disappeared. The first fasting period was one week after operation and was carried out by placing screen floors about 2 inches from the bottom of the cages. A solid surface is not suitable since it permits the ingestion of feces which might be a source of calcium. Each animal was carefully inspected at weekly intervals 24 hours after the enforcement of fasting.

In order to establish a standard by which degrees of tetanic manifestations could be measured the animals were divided into 4 classes. Tetany of degree 1 meant a hypertonicity which did not permit the animal to move rapidly or was indicated by a fine tremor. Degree 2 was indicated by sufficient hypertonicity to cause complete loss of function of one leg. Degree 3 included complete loss of function of two or more legs, and degree 4 meant that

the animal was in a state of generalized convulsions. Such a classification has many possible discrepancies but the total number of observations present a significant result. As soon as the inspection was complete the screens were removed which separated the animals from food. All animals eagerly sought food and after a few hours little or no tetany could be elicited no matter how severe it had been during the fasting period.

The above procedure was repeated at intervals of 7 days for 9 to 15 weeks. No attempt was made to control the temperature, however, the experiment began in September and was completed in May. During this period the temperature did not exceed 80° F. About an equal number of males and females were used. Litter mates were used as controls. One month after the close of the experiment 58 animals were autopsied and the neck regions removed and sectioned serially. Fifteen of these were normal controls.

RESULTS

At the end of the 7-day recovery period no tetany could be elicited in any rat. All were then subjected to 24 hours of fasting. After this fast-

TABLE 1. SUSCEPTIBILITY OF ALBINO RATS IN DIFFERENT AGE GROUPS TO TETANY IN THE FIRST 24 HOURS, AND ALSO THE EFFECTS OF 24 HOURS OF FASTING AT INTERVALS OF ONE WEEK

	Age at op. 30 days Total no. animals 26						Age at op. 60 days Total no. animals 45						Age at op. 120 days Total no. animals 21						Age at op. 180 days Total no. animals 15					
	Number of Animals						Number of Animals						Number of Animals						Number of Animals					
Week after Op.	No tetany	Degree 1 tetany	Degree 2 tetany	Degree 3 tetany	Degree 4 tetany	Dead	No tetany	Degree 1 tetany	Degree 2 tetany	Degree 3 tetany	Degree 4 tetany	Dead	No tetany	Degree 1 tetany	Degree 2 tetany	Degree 3 tetany	Degree 4 tetany	Dead	No tetany	Degree 1 tetany	Degree 2 tetany	Degree 3 tetany	Degree 4 tetany	Dead
1	0	6	6	6	7	1	7	27	7	3	0	1	8	12	1	0	0	0	10	5	0	0	0	0
2	0	9	7	7	1	1	5	21	12	3	0	3	8	11	2	0	0	0	12	3	0	0	0	0
3	0	7	8	6	1	2	9	23	5	4	0	0	9	10	2	0	0	0	12	3	0	0	0	0
4	0	11	5	5	1	0	5	22	7	5	1	1	13	7	1	0	0	0	10	5	0	0	0	0
5	1	11	7	2	0	1	14	20	6	0	0	0	12	9	0	0	0	0	10	5	0	0	0	0
6	1	12	5	2	0	1	16	18	2	2	2	0	9	11	1	0	0	0	12	3	0	0	0	0
7	0	12	4	3	1	0	13	19	7	0	0	1	10	11	0	0	0	0	12	3	0	0	0	0
8	0	12	7	1	0	0	19	14	6	0	0	0	11	9	1	0	0	0	12	3	0	0	0	0
9	7	6	6	1	0	0	17	15	5	0	1	1	17	3	1	0	0	0	11	4	0	0	0	0
10	5	10	3	2	0	0	19	15	4	0	0	0	13	8	0	0	0	0						
11	6	11	3	0	0	0							15	5	0	0	0	0						
12	6	11	3	0	0	0							14	5	1	0	0	0						
13	11	7	2	0	0	0							15	5	0	0	0	0						
14	10	8	2	0	0	0																		
15	12	6	2	0	0	0																		

ing period the animals were carefully tested and the degree of tetany recorded (table 1). Tetany was present in every animal that was operated at 30 days of age (30-day group) but was not observed in 15, 38, and 66% respectively of animals operated at 60, 120 and 180 days of age. No deaths occurred except in the 30 and 60-day groups. The severity of tetany was markedly less in the older age groups. Fourth degree tetany was present in 27% of the 30-day animals but not present in any others. Third degree tetany

was confined to the 30 and 60-day groups, 23% in the former and 7% in the latter. Several animals in the 60 and 120-day age groups showed low degree tetany because these older animals were very resistant to the development of third and fourth degree tetany. Many animals in the 180-day group showed no tetany at all and none exhibited more than the first degree.

In general there was a progressive decline in the severity of the tetany in all animals at each inspection period. At the end of the ninth weekly inspection a comparison of all groups showed that 35% of the 30-day, 43% of the 60-day, 80% of the 120-day and 73% of the 180-day animals survived a 24-hour fasting period without detectable tetany. At the end of 15 weeks, observations were made on the 30-day group, at which time second degree tetany was present in 10% of the animals.

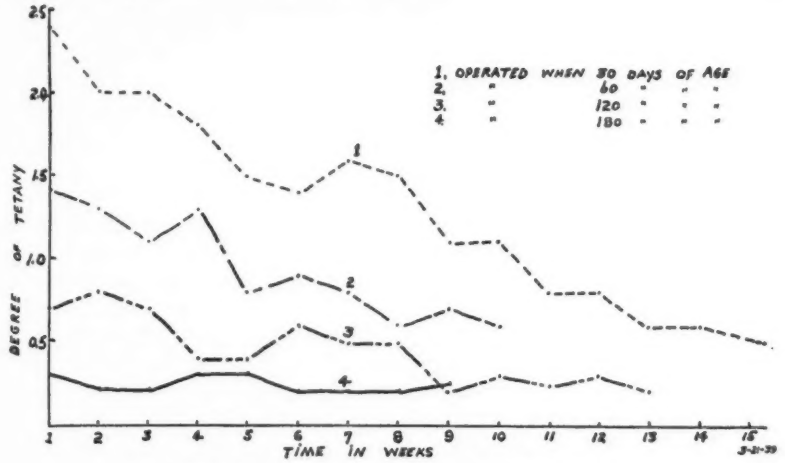

Fig. 1. Diminishing tetany in different age groups in parathyroidectomized albino rats.

In order to compare the severity of tetany in each age group the average severity for each group was obtained by multiplying the number representing the severity by the number of animals exhibiting that degree, and dividing the product by the number of surviving animals in the age group (fig. 1). At the first observation the average severity of tetany in the 30 and 180-day animals was 2.4 and 0.3 respectively. The diminishing tetany (ordinate) with increase in age (abscissa) is thus shown graphically. At the final observations (fifteenth week) the severity of tetany was 0.5 in the 30-day age group of animals as compared to 0.2 in the 120-day age group 13 weeks after operation. In the 60 and 180-day groups, 10 and 9 weeks respectively after the operation, the average severity of tetany was 0.6 and 0.25.

During the course of this experiment the total mortality was 12 animals, 8 of which died during the first week following parathyroidectomy.

In order to observe the completeness of operation all tissue removed was sectioned serially and examined histologically. Two months after the experiment ended 58 of these animals were autopsied and their neck regions sec-

tioned serially and a diligent search made for remnants of parathyroid tissue. A small amount was found in one normal control and in 4 experimental animals. In one animal it was found in the strap muscles of the neck and in another it was cephalad to the thyroid. In addition to the above, no accessory tissue was found in the neck regions of 10 females that had borne one or more litters.

DISCUSSION

The function of accessory tissue is probably the same as that of the normal glands. In the course of this and other experiments one of us (6) has observed that animals having remnants of accessory tissue seem as susceptible to tetany and as likely to die as other animals in which no residual parathyroid cells could be demonstrated. This may indicate that there is a qualitative difference between the function of accessory tissues and the normal gland but it seems more likely that differences are to be explained on the basis of quantity. To eliminate confusion and doubt it is desirable to section all tissue removed and also to examine the neck regions at the conclusion of experiments as a final check. Even with all these precautions a small error is probable, however, this error is not sufficient to vitiate results in a large and well-controlled series.

Reference to figure 1 and table 1 shows that there is a progressive adjustment of the parathyroidectomized animal. Those operated at 30 days of age required 9 to 12 weeks before most of them became normal or manifested only slight tetany after an enforced fast of 24 hours. Those operated at 60 days required only 4 to 6 weeks to reach a comparable stage of adjustment. Animals in 120 and 180-day age groups seldom showed more than slight parathyroid tetany. What is the mechanism by which this adjustment is reached? A possible explanation lies in the fact that, as was observed previously (7), there is a definite tendency for the blood plasma calcium to gradually rise to a low normal. The younger the animals the greater the time required to make the adjustment.

SUMMARY

Withdrawal of food for 24 hours induces tetany in a large percentage of parathyroidectomized albino rats after a recovery period of one week. Parathyroid tetany is not likely to be severe and very seldom causes death except in rats under 60 days of age. If rats are fasted at weekly intervals after parathyroidectomy, a progressive decline in the degree of tetany is observed.

Accessory parathyroid tissue does not vitiate experimental results in albino rats if adequate precautions are taken. Mortality is small following parathyroidectomy even in rats 30 days of age at the time of parathyroidectomy provided food is readily accessible.

It is a pleasure to acknowledge invaluable aid from Doctor Templeton of Loyola Medical School.

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REPRESSION AND RESORPTION OF THE CORPORA LUTEA OF EARLY PREGNANCY FOLLOWING INJECTIONS OF TESTOSTERONE PROPIONATE[1,2]

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IN RECENT experiments concerning the influence of testosterone propionate on transport of tubal ova in mice (1), it was observed that the corpora lutea were definitely repressed and eventually resorbed. The rate of deterioration depended upon the amount of androgen injected daily and the total number of such injections.

Mated animals were used and the first subcutaneous injections were given 10 to 14 hours after finding the vaginal plug (a.v.p.) since it was thought that early injections might interfere with the fertilization of ova. The use of mated animals is a decided advantage since this physiological phase of the ovarian cycle gives the experiment a definite starting point. This is particularly true if ova are found in the oviduct or uterus in those mice having indistinct corpora lutea.

RESULTS

Two groups of animals are reported here, one of which received 5 mg. testosterone propionate daily, while the other group had 0.25 mg. every 24 hours. Autopsies were performed 4 to 10 days after mating.

Our observations on the corpora lutea did not, at first, seem to be in line with those of Wolfe and Hamilton (2) who used 0.5 mg. testosterone propionate daily for 10 days and found larger ovaries and moderately enlarged corpora lutea in rats. On the contrary, the corpora lutea of our animals were often difficult to distinguish, especially after daily injections of 5 mg. for 8 to 10 days.

After 4 daily injections of 5 mg., we found pearly-white corpora albicans visible to the unaided eye which, at first ,were taken to be those of the preceding ovulation. The only rounded, slightly vascular bodies which were seen on the surface of the ovary did not correspond to the number of ova recovered. Not until these vascular areas were opened and ova extruded were we convinced that these corpora albicans were actually the suppressed

[1] These experiments were made possible by a grant from the Penrose Fund of the American Philosophical Society and were administered through the courtesy of Mr. C. F. Randolph, Treasurer of Alfred University.

[2] The testosterone propionate was furnished through the courtesy of Doctors Gregory Stagnell and Max Gilbert of the Schering Corporation, and Dr. R. MacBrayer of the Ciba Pharmaceutical Products, Inc.

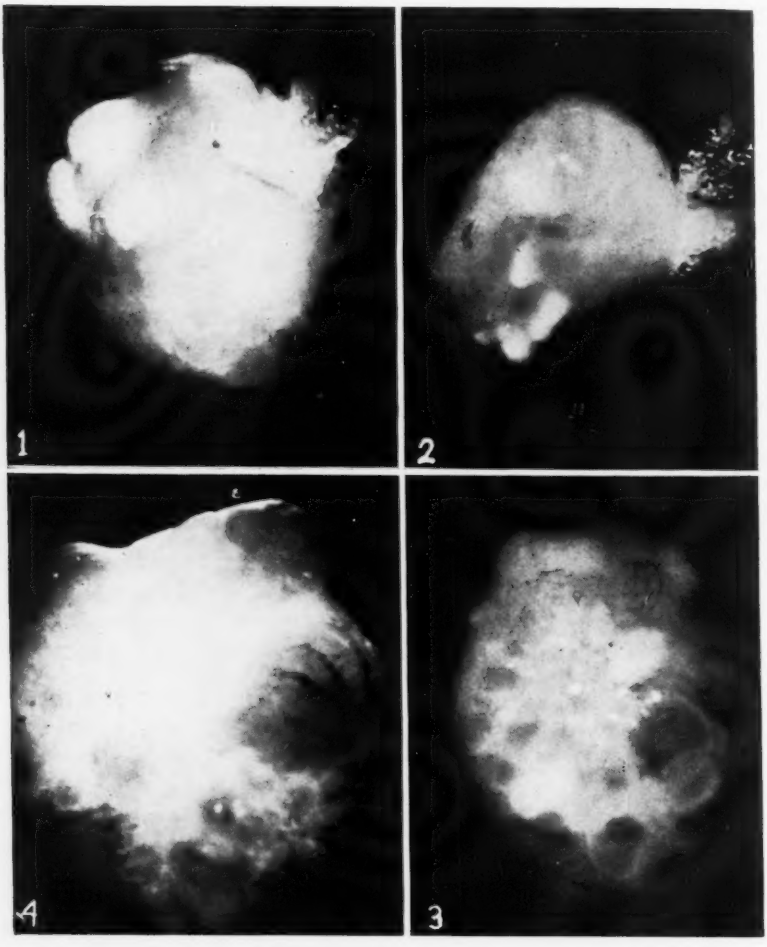

Fig. 1. Whole ovary from *mouse* 79. This animal had received 5 mg. testosterone propionate daily for 3 days and was autopsied 4 days a.v.p. Note the 3 prominent rounded corpora albicans. ×23.

Fig. 2. Whole ovary from *mouse* 81. This animal had received 5 mg. testosterone propionate for 6 days and was autopsied 8 days a.v.p. Note the 5 small irregular corpora albicans, the small size of the ovary and the absence of prominent follicles. ×23.

Fig. 3. Whole ovary from *mouse* 82. This animal had received 0.25 mg. testosterone propionate daily for 7 days and was autopsied 8 days a.v.p. Note the 2 irregular white corpora albicans and large dark follicles. Compare the size and appearance of the ovary with that of mouse no. 81. ×23.

Fig. 4. Whole ovary from pregnant mouse 18 days a.v.p. This has been included for the purpose of comparison of the corpora lutea under the same lighting conditions. Compare the dark vascular appearance of normal corpora lutea with the white corpora shown in figure 1. ×23.

corpora lutea of this last ovulation. The number of corpora albicans usually matched the total of ova recovered. Figure 1 is a photograph of a whole ovary from *mouse* 79 which had received 3 daily injections of 5 mg. testosterone propionate and which was autopsied on the fourth day. Note the cluster of 3 prominent white corpora albicans which are characteristic in appearance for the first stage of degeneration. That this is not just a photographic effect can be seen by comparing these corpora with those of figure 4 which are of late pregnancy.

It then seemed probable that testosterone propionate could not only inhibit the growth of the corpus luteum but hasten its resorption since it had been shown by Wolfe and Hamilton (2) that the gonadotropic capacity of pituitaries from androgen-treated animals was greatly decreased. Our subsequent experiments support this conclusion. After 5 to 6 days of 5 mg. injections, the sharp line of demarcation between the corpora albicans and the body of the ovary is lost and the corpora show changes in outline. Whereas they were at first round, as in figure 1, some of the corpora are now narrow along one axis and somewhat rectangular in appearance. Figure 2 is a photograph of a whole ovary showing such a condition. The animal (No. 81) furnishing this ovary had received 5 mg. daily for 6 days and was autopsied on the eighth day a.v.p. In the photograph the corpora albicans stand out a little too sharply on what appears to be the edge of the ovary. This exaggeration is due to the angle at which the ovary was lighted and also to the absence of back lighting on the specimen. After 8 days of such injections the corpora lutea have changed to corpora albicans and are usually reduced to such small irregular white spots that they are often impossible to distinguish. The ovaries of all animals receiving 5 mg. for 8 days are markedly reduced in size, hypoemic and the follicles are inconspicuous.

Having determined the effect of relatively large doses of this androgen, it was decided to use only 0.25 mg. daily. The ovaries of this series differed in appearance from those of the first group. They showed increased size yet were rather translucent due to the many enlarged follicles. The corpora lutea of animals autopsied on the fourth day a.v.p. after having received 3 daily injections of 0.25 mg. are not particularly blanched nor are they yet reduced in size. However, when autopsied on the sixth day, after 5 such injections the corpora lutea were definitely whiter. Figure 3 is the photograph of the left ovary of animal No. 82 which had received 7 injections of 0.25 mg. and was autopsied on the eighth day, 182 hours a.v.p. The corpora lutea show definite effects of the androgen, being white, smaller in diameter and irregular in shape. The narrowing along one axis is typical of the corpora lutea response in these experiments. The darker areas in figure 3 are enlarged follicles and the minute white spots which look like high-lights on the ovary are remnants of corpora albicans of preceding ovulations.

Although the effects of testosterone propionate on the tubal passage of ova and on pregnancy will be reported at another time, it should be indicated here that either dose prevents pregnancy. However, such sterility is only temporary as the ovary soon recovers its ovulational capacity.

DISCUSSION

In checking over the literature on the effects of injections of androgens on ovaries, we found considerable confusion in the interpretations of results. This is more apparent than real since most of the experiments are not parallel in procedure. Furthermore, the conclusions are sometimes too inclusive. For one to say that testosterone propionate causes an increase or a decrease in ovary weight is not sufficient. The time-dosage factors must be clearly shown and the stage in the estrous cycle at the beginning of the experiment should be stated. The accompanying table is a summary of some of the earlier

TABLE 1. SUMMARY COVERING THE EFFECTS OF TESTOSTERONE PROPIONATE ON CORPORA LUTEA

Experimenters	Injections Amount	Injections No. Days	Animals Used	Condition of Corpora Lutea
Boling & Hamilton (3)	4.0 mg.	2–6 Others 10–18	Guinea pigs	Follicles arrested and small in size.
Courrier & Gros (4)	10.0 mg.		Rabbits	Atrophy of c.l. in early pregnancy.
Freed et al. (5)	(1) 0.05 mg.	daily	Rats	(1) Few follicles; c.l. absent
	(2) 1.0 mg.	16 days	Rats	(2) Numerous large c.l.
Hamilton & Wolfe (6)	0.5 mg.	15–30	Rats	
Korenchevsky et al. (7)	1.5 mg.	21	Rats	Increase in size and number of c.l.
McKeown & Zuckerman (8)	0.2 mg.	10	Rats	Increase in size of c.l. but no large follicles; c.l. are of various sizes and some degenerating.
Mazer & Mazer (9)	0.5 mg. 3× weekly	62–68	Rats	c.l. absent; arrest of follicles.
Wolfe & Hamilton (2)	0.5 mg.	10	Rats	Moderately enlarged c.l. only in animals at estrus or metestrus at beginning of experiments.
Zuckerman (10)	25.0 mg. 2× weekly	210	Monkey	No c.l.; follicles very small.

experiments which mention the effect of testosterone propionate on corpora lutea of adult animals. It will be noted that the corpora lutea discussed in this summary are not, except in one instance (4), those of a known ovulation.

Our results would seem to differ radically from those of Freed, Greenhill and Soskin (5) who treated rats for 16 days with testosterone propionate and found numerous enlarged corpora lutea with 1 mg. doses but no corpora lutea with 0.05 mg. injections. The explanation probably rests with the dosage-weight relationship since our largest injections were 5 mg. for mice while theirs were only 1 mg. for rats. Then, too, their animals were injected 16 days without any apparent relationship to a particular set of corpora lutea. Nor is it known how the 1 mg. injections in rats affected the ovaries through-

out the 16-day period. It is quite possible that this amount stimulated the pituitaries to secrete gonadotropic hormone with results comparable to the 0.25 mg. injections in mice but with this exception: the corpora lutea in the experiments were those of early pregnancy while those reported by Freed, Greenhill and Soskin were from unmated animals.

On the other hand, there may be in rodents definite levels at which testosterone propionate affects the pituitary differently. The inhibition of the rat ovary after the 0.05 mg. may possibly produce similar results in the mouse with smaller injections than the 0.25 mg. which we used. And again, if Freed et al. had used 5 to 10 mg. for another series of rats, might there not have resulted a decrease in size of the corpora lutea and the ovaries as was found after 5 mg. doses in mice? In other words, it is quite possible that both small and large doses depress the activity of the pituitary while an intermediate dose relatively stimulates that gland. Such factors should be determined before clinical application can be made of the androgens. Hamilton and Wolfe (6) have expressed the opinion that results of testosterone propionate injections may be explained as being due to either suppression of the production of the gonadotropic hormone or the release of that hormone stored in the anterior pituitary. Since we find larger ovaries after several days of small injections, some gonadotropic factor is apparently released. On the other hand, even these smaller injections, when continued for several days, appear to inhibit the secretion of the luteinizing hormone of the pituitary. This is indicated by the changes in color and size of the corpora lutea.

After several 5 mg. injections both the corpora lutea and the ovaries are soon reduced in size which would indicate the suppression of both the follicle-stimulating and the luteinizing hormones of the pituitary. Whether there is an initial stimulation within the first few hours has not yet been determined. To Mazer and Mazer's (9) conclusion that duration of treatment is important in determining the effect of testosterone propionate on the ovaries, we would also add the equally significant dosage-factor. In experiments concerning the effects of androgens on corpora lutea in mice and rats, it is of utmost importance to know just what series of corpora lutea one is examining. The use of mated animals, checked for ova, obviates this difficulty and gives the experimenter an excellent physiological starting point.

Furthermore, it seems quite possible to use the rate of repression and resorption of corpora lutea of early pregnancy for the determination of the quantity or potency of testosterone propionate present in unknowns. The rabbit might also be used since Courrier and Gros (4) found that the injection of 10 mg. testosterone propionate led to the atrophy of the corpora lutea of early pregnancy in rabbits.

SUMMARY

In mice, daily injections of 5 mg. testosterone propionate begun the day of mating cause the corpora lutea to become corpora albicans and hasten their resorption. These corpora are barely discernible after 8 days of such injections. The ovaries are decreased in size.

Similar treatment of mice with 0.25 mg. for the same length of time results in larger ovaries but in small and degenerated corpora lutea. While their resorption is not as rapid as in the 5 mg. series, the final changes are just as definite. Temporary sterility is established with either amount. The rate of resorption of corpora lutea is proposed as a possible means for determining the amounts of testosterone propionate present in unknowns.

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DETERMINATION OF CRYSTALLINE ANDROSTERONE WITH THE EVELYN PHOTOELECTRIC COLORIMETER

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THE ZIMMERMANN procedure for color development in an alkaline alcoholic solution with substances containing the $-CH_2CO$ grouping has been applied by a number of investigators to the assay of crystalline and urinary androgens (1–7). Our modification of this reaction for use in the Oesting colorimeter has been found to be of distinct clinical value in the assay of urinary androgens (7). These observations were extended to include a similar study with the Evelyn photoelectric colorimeter (8) in order to eliminate the subjective factors inherent in visual comparative measurements and because one can study under more favorable circumstances the factors which influence the rate and course of color development. For these reasons the instrument was useful in the development of a more accurate method for the determination of ketonic sterols such as crystalline androsterone and dehydroisoandrosterone. This communication is concerned with the method of color development and the calibration of the Evelyn photoelectric colorimeter for this technic with crystalline androsterone. Similar studies are being carried out with crystalline dehydroisoandrosterone and the method has been applied also to the quantitative determination of the total urinary ketonic sterols contained in the neutral fraction of the extract.

Several factors came up for consideration in the adaptation of the Zimmermann reaction for use in the Evelyn photoelectric colorimeter.

(*a*) Selection of the proper filter (No. 520) by spectrophotometric analysis of alcoholic solutions of crystalline androsterone (fig. 1).

(*b*) Care in the purification, standardization and storage of reagents.

(*c*) Standardization of the variable factors which influence the rate and course of color development. Since the factors which contribute to the intensity of the pinkish color of the test solution also affect the color of the blank solution, one must adjust these variables so that the color of the test solution is as deep as possible while the blank solution remains as nearly colorless as possible. This was done without sacrificing the constancy of the results.

(*d*) Determination of the upper and lower limits of the galvanometer scale within which, in accordance with Beer's law, the depth of color produced is a linear function of the amount of androgen present.

Minor changes in our original modification of the Zimmermann procedure for use in the Oesting colorimeter were found to be necessary due

[1] Aided by a grant from the Milton Fund, Harvard University.

[2] We are indebted to Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc., and to Dr. Erwin Schwenk of the Schering Corporation for the crystalline androsterone used in these experiments.

to the high sensitivity of the Evelyn photoelectric colorimeter in comparison with the Oesting instrument.

Procedure for color development with crystalline androsterone. Calibrated 1 cc. pipettes graduated to 0.01 cc. were used for the measurements of all reagents and solutions to be assayed. A standard stock solution of 100 cc.

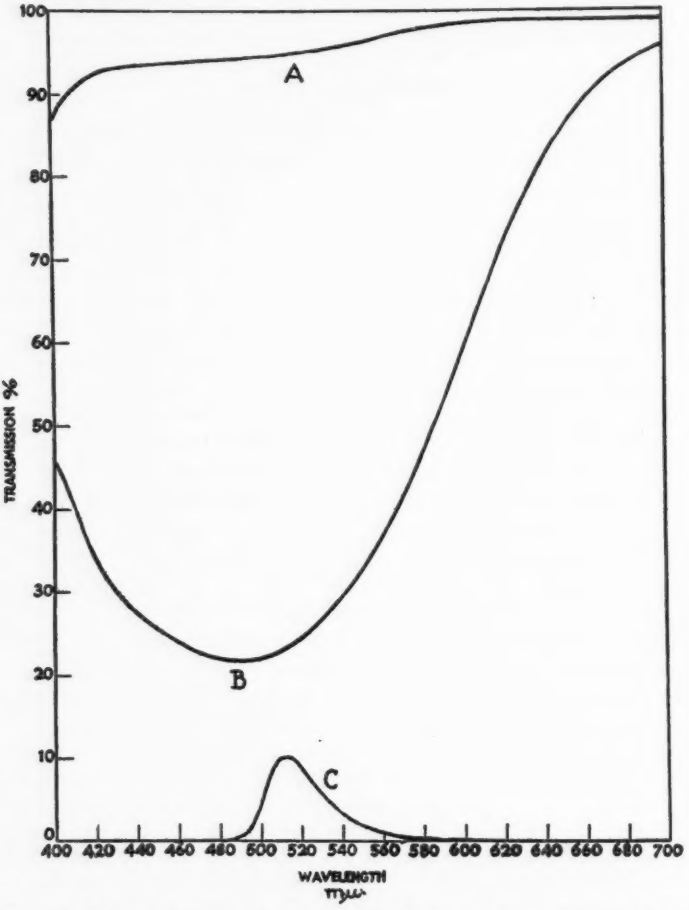


Fig. 1. A. Spectrophotometric curve for the blank solution containing reagents only. B. Spectrophotometric curve for the color developed in solution with androsterone by the modification of the Zimmermann reaction described in this paper. C. Spectrophotometric curve of No. 520 filter of the Evelyn photoelectric colorimeter.

was prepared so as to contain 1.5 mg. of androsterone per cc. of 95% ethyl alcoholic solution. Two aliquots of this standard solution were diluted to a volume of 100 cc. with 95% ethyl alcohol. The aliquots were chosen so that 1 cc. of the new solutions contained 0.75 and 0.375 mg. of androgen, re-

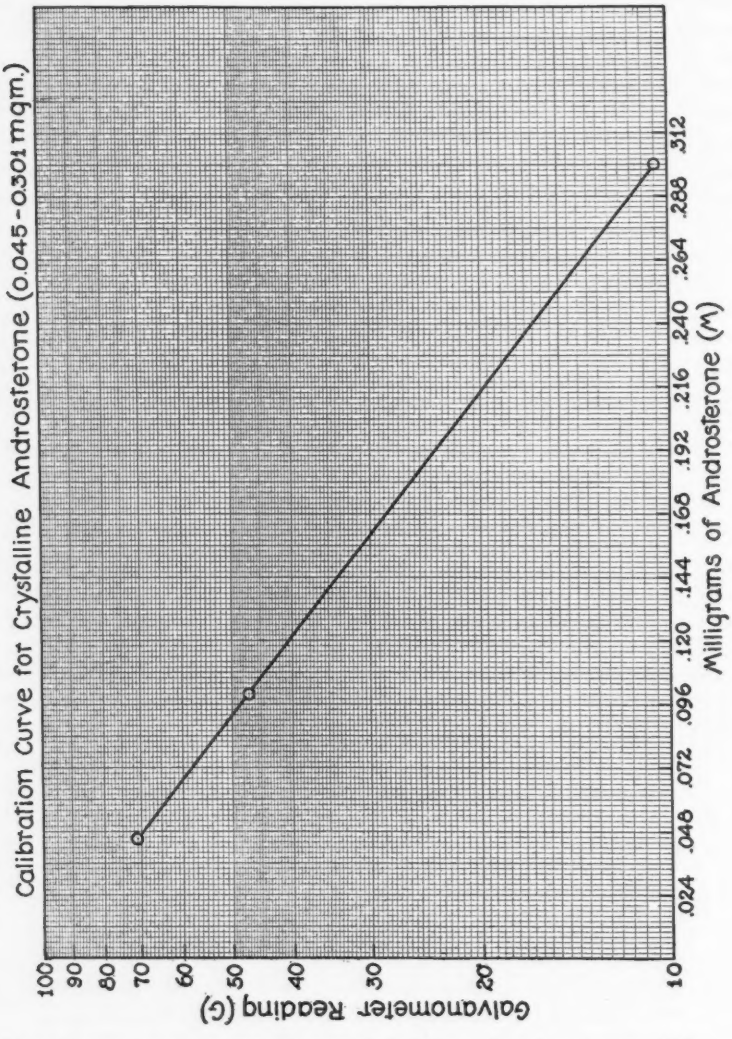
spectively. Two-tenths of a cc. of these standard solutions, or smaller aliquots diluted to 0.2 cc. with 95% ethyl alcohol, were accurately measured into the colorimeter absorption tubes. Care must be taken not to lose any of the solutions on the walls of the tube. To each was added with the same precautions, 0.2 cc. of 2% m-dinitrobenzene reagent in absolute ethyl alcohol and 0.2 cc. of 2 N aqueous potassium hydroxide. A blank solution containing the reagents and the 0.2 cc. of 95% ethyl alcohol was prepared at the same time. All determinations were set up in duplicate. After the contents of the tubes were thoroughly mixed they were covered and kept for 1¼ hours with frequent shaking in a water bath which was thermostatically controlled at 25°C. At the end of this time dilution to a total volume of 10.6 cc. was made with 10 cc. of 95% ethyl alcohol. The final solutions were then thoroughly mixed and the depth of the pink color was read immediately in the Evelyn photoelectric colorimeter.

Determinations in the colorimeter are made with the 10 cc. aperture and with the filter No. 520 in place. The reading of the blank solution, which contains only the reagents and alcohol, is adjusted to 100 on the galvanometer scale. The galvanometer reading obtained after removal of the blank solution becomes the center setting from which subsequent readings are made on all test solutions. A good check on the freshness and purity of the reagents is obtained by watching the center setting of the blank tube from day to day. This center setting has been found to vary only from 81[1] to 82[2] using different batches of carefully purified and freshly prepared reagents over a 5-month period. The center setting rises above 82[2] and greater variability in the results is obtained with duplicate test determinations unless these conditions are scrupulously fulfilled. Ordinarily determinations on duplicate test solutions vary only by 1% with an occasional difference as great as 3%.

Formula and calculation used in calibration. In accordance with the laws of Lambert and Beer, the formula $C = L/K_1$ is applicable to the data in table 1 which shows that, within the limits imposed by the instrument and the technic, the concentration of the colored compound is proportional to the negative logarithm of the transmission of light. In other words, the depth of color developed in the reaction mixture is directly proportional to the amount of androsterone present in solution. In this formula C represents the amount of androsterone per cc. of colored solution and is numerically equal to M/10.6 where M is the amount in mg. of androsterone in the total volume (10.6 cc.) of colored solution. K_1 is a constant whose value depends on the nature of the colored solution and the characteristics of the filter used for the determination. L, which is measured by the negative logarithm of the transmission of light ($2 - \log G$), represents the density of the solution.

Four different but identically prepared batches of the stock standards and reagents were used in the calibration. Calibration consisted in determining the value of K_1 by the above formula for known concentrations of androsterone in alcoholic solution. Such determinations were made in duplicate and checked several times with various concentrations of this androgen

within the galvanometer range of 10 to 90. The K_1 values for amounts of androsterone ranging from 0.019 to 0.045 mg. show greater variability than those derived from 0.045 to 0.301 mg. The latter range was chosen, therefore, for the determination of the mean K_1 value, and concentrations of solutions



with unknown amounts of androgen were adjusted so that they could be read within the range of the galvanometer readings corresponding to these limits.

The mean value of K_1 in the range of 0.045 to 0.301 mg. of androsterone is 34.72 ± 0.68. This represents a coefficient of variation from the mean of $\pm 1.96\%$.

TABLE 1

M	G	L	C	K_1	M	G	L	C	K_1
0.019	87^1	0.0593	0.0018	32.94	0.075	57^0	0.2441	0.0071	34.38
0.019	87^2	0.0568	0.0018	31.56	0.075	56^1	0.2499	0.0071	35.20
0.020	88^1	0.0543	0.0019	28.58	0.075	57^0	0.2441	0.0071	34.38
0.020	86^2	0.0630	0.0019	33.16	0.075	57^2	0.2403	0.0071	33.85
0.020	87^2	0.0580	0.0019	30.53	0.075	56^0	0.2518	0.0071	35.47
0.020	86^1	0.0642	0.0019	33.79	0.075	55^3	0.2537	0.0071	35.73
0.020	86^0	0.0655	0.0019	34.47	0.075	56^2	0.2480	0.0071	34.93
0.020	87^0	0.0605	0.0019	31.84	0.075	56^1	0.2499	0.0071	35.20
0.020	86^1	0.0642	0.0019	33.79	0.077	56^0	0.2518	0.0073	34.49
0.020	87^2	0.0580	0.0019	30.53	0.077	56^3	0.2460	0.0073	33.70
0.026	82^3	0.0822	0.0025	32.08	0.113	42^1	0.374	0.0107	34.95
0.026	82^1	0.0848	0.0025	33.92	0.113	42^3	0.369	0.0107	34.49
0.026	81^1	0.0901	0.0025	36.04	0.113	42^2	0.369	0.0107	34.49
0.026	81^1	0.0901	0.0025	36.04	0.113	41^3	0.380	0.0107	35.51
0.026	86^1	0.0642	0.0025	25.68	0.113	42^3	0.369	0.0107	34.49
0.030	79^1	0.1010	0.0028	36.07	0.113	44^0	0.357	0.0107	33.36
0.030	80^3	0.0928	0.0028	33.14	0.113	42^2	0.372	0.0107	34.77
0.030	80^3	0.0928	0.0028	33.14	0.115	41^0	0.387	0.0109	35.50
0.030	80^3	0.0928	0.0028	33.14	0.115	41^3	0.382	0.0109	35.05
0.030	80^3	0.0928	0.0028	33.14	0.151	32^3	0.485	0.0142	34.16
0.030	81^0	0.0915	0.0028	32.68	0.151	33^0	0.482	0.0142	33.94
0.030	84^2	0.0731	0.0028	26.11	0.151	31^1	0.505	0.0142	35.56
0.030	83^3	0.0770	0.0028	27.50	0.151	31^3	0.498	0.0142	35.07
0.037	73^3	0.1322	0.0035	37.77	0.153	30^1	0.520	0.0144	36.11
0.037	73^0	0.1367	0.0035	39.06	0.153	31^2	0.502	0.0144	34.86
0.037	77^1	0.1121	0.0035	32.03	0.226	17^0	0.770	0.0213	36.15
0.037	77^0	0.1135	0.0035	32.43	0.226	18^2	0.733	0.0213	34.41
0.039	74^2	0.1278	0.0037	34.54	0.256	14^3	0.831	0.0242	34.34
0.039	74^1	0.1293	0.0037	34.95	0.256	15^0	0.824	0.0242	34.05
0.039	75^0	0.1249	0.0037	33.76	0.256	14^0	0.854	0.0242	35.29
0.039	78^1	0.1065	0.0037	28.76	0.256	14^3	0.831	0.0242	34.34
0.039	78^2	0.1051	0.0037	28.46	0.256	14^1	0.846	0.0242	34.96
0.039	73^3	0.1322	0.0037	35.73	0.256	15^2	0.810	0.0242	33.47
0.039	74^2	0.1278	0.0037	34.54	0.256	14^2	0.838	0.0242	34.63
0.039	73^0	0.1367	0.0037	36.95	0.260	14^1	0.846	0.0245	34.53
0.039	73^3	0.1322	0.0037	35.73	0.260	13^3	0.862	0.0245	35.18
0.045	70^1	0.1534	0.0043	35.67	0.271	13^0	0.886	0.0256	34.61
0.045	71^2	0.1457	0.0043	33.88	0.291	11^2	0.939	0.0275	34.15
0.045	70^2	0.1518	0.0043	35.30	0.291	11^1	0.949	0.0275	34.51
0.045	71^1	0.1472	0.0043	34.23	0.301	10^1	0.989	0.0284	34.82
0.045	70^3	0.1503	0.0043	34.95	0.301	10^2	0.979	0.0284	34.47
0.045	70^1	0.1534	0.0043	35.67	0.301	10^3	0.969	0.0284	34.12
0.045	72^0	0.1427	0.0043	33.19	0.301	10^0	1.000	0.0284	35.21

M: Amount in mg. of androsterone in the total volume (10.6 cc.) of colored solution.

G: Corrected galvanometer reading (i.e., corrected for the slight individual variations of different galvanometers from true linearity).

L: Measured by the negative logarithm of the transmission of light ($2-\log G$) and represents the density of the solution.

C: Amount of androsterone in mg. per cc. of colored solution and is numerically equal to $\frac{M}{10.6}$

K_1: A constant which is numerically equal to $\frac{L}{C}$.

A calibration curve (fig. 2) was constructed from the data in table 1. The best fitted line for these data is represented by the regression equation $L = 2.27M$ with a residual standard deviation of 0.019. The calibration curve itself represents M, milligrams of androsterone in the total volume of colored solution, plotted on a logarithmic scale against the galvanometer readings, which correspond to L values in the above equation. These values are pro-

portional to C and L, respectively, which were used in the calculation of K_1. The data were plotted in terms of the aforementioned values instead of C and L because evaluation of unknowns directly from a calibration curve based on observed galvanometer readings is much more convenient for routine purposes.

Practical use of the calibration values with crystalline androsterone. Amounts of androsterone giving readings at the two extremes and near the middle of the calibration curve were used to determine the limits of experimental error in the assay of the androgen from either the value of K_1 or from the calibration curve itself. Table 2 contains a summary of the results of these observations which were made over a period of several months with different batches of reagents.

TABLE 2

Androsterone known to be in solution	No. of determinations	Androsterone detected in solution	Standard coefficient of variation from mean value	Percentage standard error from known amount of androsterone
		Mean value calculated from K_1 or calibration curve	%	
mg.		*mg.*		
0.045	22	0.046±0.002	4.3	2.20
0.151	21	0.152±0.004	2.6	0.66
0.301	20	0.302±0.005	1.7	0.32

SUMMARY

A modification of the Zimmermann procedure for color development has been applied to the assay of crystalline androsterone, and the Evelyn photoelectric colorimeter has been calibrated for the technic in terms of androsterone. The greatest advantage gained by the use of this colorimeter is the elimination of the subjective factor inherent in visual comparative measurements. The data obtained indicate a high degree of reliability and reproducibility of results in the assay of crystalline androsterone.

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HISTOLOGICAL CHANGES IN THE SENILE VAGINA INDUCED BY ESTROGENIC THERAPY ADMINISTERED ORALLY AND BY INUNCTION[1]

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THE CYCLICAL CHANGES in the human vaginal mucosa have been demonstrated by Dierks (1, 2, 3) in 1927, 1929 and 1930, by Geist (4) in 1930 and Papanicolaou (5) in 1933 and Papanicolaou and Shorr (6) in 1936. With the advent of the menopause the vaginal mucous membrane gradually regresses to an inactive state in the majority of women, and the symptoms of senile vulvo-vaginitis, such as pruritus, discharge and dyspareunia may then make their appearance.

The hypodermic administration of potent estrogenic preparations in adequate dosage restores the vaginal epithelium to the state found during active sex life. This phenomenon has been demonstrated in the monkey (7, 8), in the rodent (9, 10), in the human being (2, 6, 11–15). These authors used various types of estrogenic hormones: theelin, amniotin, and progynon-B. Davis used amniotin both by injection and suppository, but he found that when given in conjunction, the combined therapy proved most satisfactory.

The effect of estrogens when applied by inunction in the rodents, primates and in the human being has been demonstrated (16–24).

Papanicolaou and Shorr (6) found that amniotin in oil from 250 to 500 R.U. given hypodermically daily or every other day for a period of about a month caused a slow, but pronounced growth of vaginal epithelium; this form of therapy also resulted in relief from menopausal symptoms such as hot flushes, nervousness and irritability, depression, asthenia and insomnia. Progynon-B, when given hypodermically in doses of 10,000 R.U. every third or fourth day over a period of approximately one month (until about 70,000 R.U. were given) caused a more rapid growth of vaginal epithelium, but a less sustained relief from menopausal symptoms. Oral administration of estrogenic hormone (amniotin in oil) in the same dosage as given by injections was less effective in relieving the symptoms or producing a typical estrous vaginal smear.

The effect of intensive estrogenic therapy in our series of 51 cases of senile vulvo-vaginitis was followed in all patients by observing the changes in the vaginal smear by the method of Papanicolaou and Shorr (6) and Richardson, Papanicolaou and Shorr (25). In 39 of these patients the effect was also observed by means of vaginal biopsies. A total of 110 biopsies was taken in the latter group.

[1] Read before the 23rd Annual Meeting of the Association for the Study of Internal Secretions in St. Louis, Missouri, May 13, 1939.

The estrogenic substances were administered by means of intramuscular injection (10 patients), vaginal suppository (4 patients), topical inunction (21 patients) and orally (16 patients). The age of the patients ranged from 35 to 75 years.

The histological changes produced in the senile vagina by estrogenic therapy administered parenterally and by suppository have already been described (6, 11–14); this paper, therefore, deals only with the response of senile vulvo-vaginitis to estrogenic hormones administered by the oral route and by inunction of the vulvo-vaginal area in 37 patients. The results of injection and suppository treatment of the 14 remaining patients will be described elsewhere. The progress of 12 patients receiving inunction therapy and 8 of the patients receiving oral therapy were studied with the aid of vaginal biopsy, the total number of biopsy studies being 68.

The vaginal specimens for biopsies were taken before, during the course of, and after cessation of therapy. Specimens for biopsies were also taken after periods of control therapy with placebos, and bland and analgesic ointments. In some instances, incomplete regression of the vaginal mucosa was noted several years after the onset of the menopause, therefore, comparative grading was made on successive biopsies, rather than absolute evaluation of the individual biopsies. In isolated instances there were small localized areas, which varied in activity from the remainder of the vaginal epithelium.

The estrogenic preparations used were as follows:

(*a*) Progynon-D-H Ointment (dihydroxyestrin lanol) for inunction[1]

(*b*) Progynon-D-H Tablets (dihydroxyestrin) for oral use[1]

(*c*) Emmenin (estriol glucuronide) for oral use[2]

(*d*) Estrobene (diethylstilbestrol) for oral use[2]

The following 5 case records exemplify the characteristic effects of estrogenic therapy administered by inunction and orally. The first 2 patients were treated by inunction, the last 3 orally. All 5 patients showed marked improvement in local symptoms such as pruritus (vulvae and vagina), dyspareunia, dryness and cracking of the skin in the vulval area, and dysuria with varying degrees of improvement in the general menopausal symptoms together with a restoration of the active vaginal epithelium. The therapy with estrobene (diethylstilbestrol) was initiated in 2 patients following the investigations by Dodds, Goldberg, Lawson and Robinson (26), and others (27–30). The onset of menopause in these two patients was 8 years and 7 months respectively. However, both patients presented symptoms of vasomotor disturbances as well as of senile vulvo-vaginitis.

As pointed out above, the majority of patients on estrogenic therapy administered orally and by inunction responded favorably to both of these types of therapy. There were, however, 6 patients in the series of 21 patients treated by inunction, who obtained partial relief only. In these patients a change to injection therapy was made without marked improvement, except

[1] Progynon-D-H Ointment, progynon-D-H Tablets and progynon-B have been supplied by the courtesy of Dr. Gregory Stragnell and Dr. Max Gilbert of the Schering Corporation.

[2] Emmenin and estrobene were supplied by the courtesy of Mr. Joseph Hutchinson of Ayerst, McKenna & Harrison.

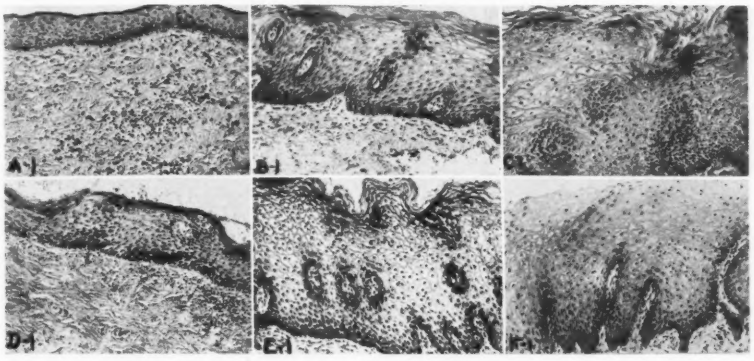

Fig. 1. Case 1. Therapy with Topical Inunction

A1 June 15, 1937. Vaginal biopsy before therapy. Note thin epithelial layer consisting of small cells and round cell infiltration in the sub-epithelial layer.

B1 July 13, 1937. Moderate development of epithelial layer. Note thickened epithelium. The cells contain an abundance of cytoplasm, the basal layer of cells show activity, the sub-epithelial tissues contain numerous capillaries.

C1 September 13, 1937. Shows advanced development of the epithelial layer with vacuolated cells in the basalis. The functionalis is beginning to appear.

D1 February 26, 1938. Involution after control period with analgesic non-estrogenic ointment.

E1 April 22, 1938. Development after instituting estrogenic therapy.

F1 May 25, 1938. Slight regression after irregular application of inunction.

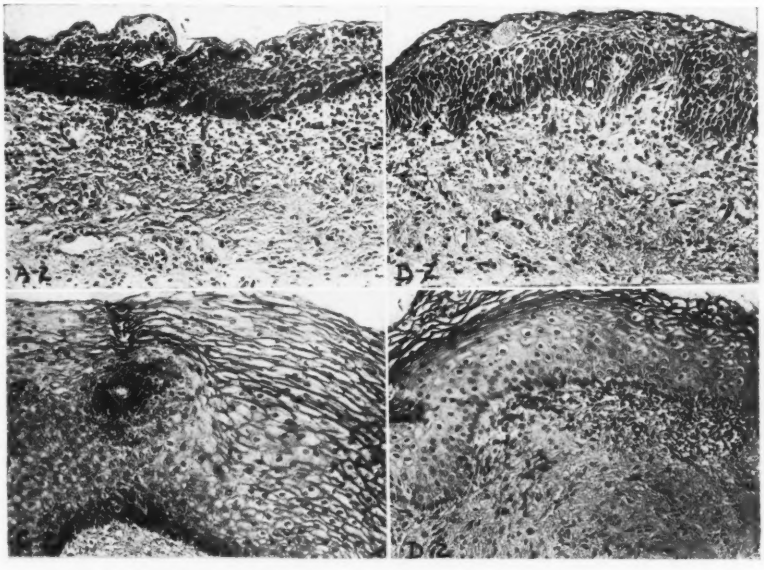

Fig. 2, Case II. Therapy with Topical Inunction

A2 March 7, 1938. Atrophic vagina with leucocytic infiltration of epithelial as well as sub-epithelial layers.

B2 March 10, 1938. After topical inunctions for 3 days showing diminution of leucocytic infiltration and marked activity of epithelial cells.

C2 April 4, 1938. Development of basalis after topical inunctions.

D2 April 26, 1938. Incomplete regression after discontinuing therapy for 10 days. Note reappearance of leucocytes in sub-epithelial zone and thinning of epithelial layer.

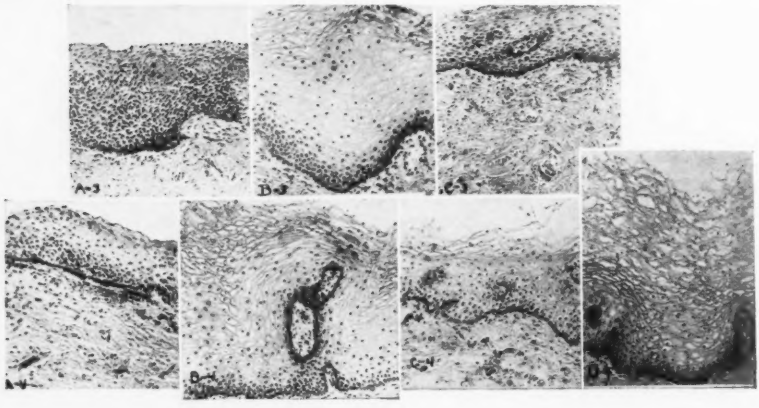

Fig. 3

Case III. Oral Estrogenic Therapy. A3 June 14, 1938. Initial biopsy before therapy. B3 October 8, 1938. Thickening of epithelial cell layer with the formation of a definite basalis. Many of the cells are filled with glycogen. Note the capillaries in the sub-epithelial tissues. C3 January 31, 1939. Regression after discontinuing therapy for 2 months.

Case IV. Injection and Oral Estrogenic Therapy. A4 November 3, 1937. Biopsy before therapy. B4 December 31, 1937. Development of epithelial layer after injection therapy. C4 April 13, 1938. Partial regression after mild oral therapy. D4 February 29, 1939. Progression after intensive oral therapy.

in one instance. It should, however, be pointed out that there were complicating factors present such as diabetes, eczema, extensive leukoplakia and hypertrophic pathological changes of the vulva. Of the 16 patients treated with oral medication, one case received partial benefit only despite intensive therapy.

Case 1, inunction therapy. B.H. age 53, had had spontaneous menopause 9 years previously. Intense itching and burning of the vulva, vaginal discharge, dermatitis and blistering of labia majora. Patient complained of intense itching; local treatment with an analgesic ointment was unsuccessful. A specimen for a biopsy obtained from the left vulvovaginal area on June 15, 1937 (fig. 1, case 1, A) showed a senile type of vaginal epithelium. For the following three months the patient was supplied with

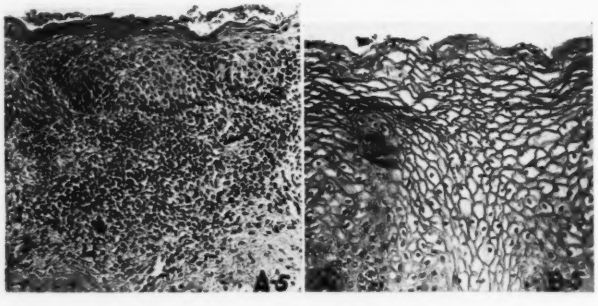

Fig. 4

Case V. Therapy with Diethyl-Stilbestrol. A5 March 28, 1939. Vaginal biopsy before therapy showing atrophic epithelial layer with considerable sub-epithelial round cell infiltration. B5 April 27, 1939. Thickened epithelial layer with vacuolated cells.

an estrogenic ointment (progynon-D-H ointment—dihydroxyestrin in lanolin) of varying strengths ranging from 2,000 R.U. to 50,000 R.U. per 2 oz. jar. The total amount used by the patient in 3 months was estimated to be 100,000 R.U. of dihydroxyestrin. During that period two additional biopsies were made on July 13, 1937 (fig. 1, case 1, B) and September 13, 1937 (fig. 1, case 1, C) each one showing a definite progression from the original senile status. There was gradual, but marked improvement in the local symptoms previously complained of and on October 10, 1937 the patient had a uterine bleeding lasting one week after a 10-year menopause. The treatment was temporarily discontinued after this episode. The symptoms began to return and an analgesic ointment was substituted for the purpose of control study. The biopsy performed on February 26, 1938 (fig. 1, case 1, D) showed a regression of the vaginal epithelium to an inactive state. On April 1, 1938 the patient was returned to the progynon-D-H ointment inunctions with ensuing relief of symptoms. Specimens for vaginal biopsies taken on April 22, 1938 (fig. E1) and May 25, 1938 (fig. 1, case 1, F) showed a restoration and growth of the vaginal epithelium. The dosage, however, was then reduced by one half, and this in all probability accounts for the slight regression in figure F1. The patient is now receiving satisfactory maintenance doses (10,000 to 14,000 R.U. per week of dihydroxyestrin in the form of an ointment). There has been no further uterine bleeding.

Case 2, inunction therapy. S.M., age 60, had had spontaneous menopause 15 years previously. Complaints were: burning urination, discharge, pain and itching in the vulvo-vaginal area. On examination the introitus appeared acutely inflamed, partially stenosed, covered with a purulent discharge. The examinations for gonorrheal infection and trichomonas vaginalis infestation were negative. A specimen for a vaginal biopsy obtained with difficulty on March 7, 1938 (fig. 2, case II, A) showed a low vaginal epithelium, and inflammatory changes in the mucous and submucous layers. The patient was given progynon-D-H ointment and instructed to apply it to the affected area, and well within the introitus. Fearing that the original biopsy may not have been entirely satisfactory, the patient was instructed to return in 3 days, at which time another biopsy was made on March 10, 1938 (fig. 2, case II, B). At that time the appearance of the vulva and introitus was markedly improved and the patient confirmed this observation by stating that there was a partial relief of the symptoms originally complained of. The histological examination showed a definite proliferative tendency on the part of the epithelial elements and a diminution of leucocytic infiltration. The treatment was continued and another biopsy made on April 4, 1938 (fig. 2, case II, C) showing a marked epithelial growth, glycogen laden cells and complete disappearance of leucocytic infiltration. The total period of treatment was three weeks and the total amount of ointment used represented 18,000 to 20,000 R.U. of dihydroxyestrin. An interruption in the treatment (10 days) resulted in a partial regression evidenced by a biopsy made on April 26, 1938 (fig. 2, case II, D). The patient has not been seen since the above date.

Case 3, oral therapy. Mrs. S.P., a white female age 41, spontaneous menopause 2 years previously, was admitted to the clinic on June 11, 1938 with symptoms of hot flushes, pruritus, dysuria and vertigo. A specimen for a biopsy made on June 14, 1938 (fig. 3, case III, A) showed moderately senile regression. The patient was placed on specially prepared emmenin (1,000 day-oral Collip units per cc.); 1 cc. was taken 3 times a day after meals for 3 weeks. The patient felt a great deal improved, but failed to return to the clinic when the medication was exhausted. She remained without treatment for one month and experienced the return of all the symptoms. On August 20, 1938 the patient was again placed on the special emmenin, this time on a

larger dosage, 2 cc. 2 or 3 times a day (4,000 to 6,000 day oral Collip units). Marked improvement was soon experienced and a vaginal biopsy on October 10, 1938 showed considerable growth of the vaginal epithelium. After this time medication was allowed to elapse for 2 months, which resulted in a recurrence of the symptoms. A specimen for a biopsy taken on January 31, 1939 (fig. 3, case III, C) again showed a moderate regression. The patient is now receiving maintenance doses of emmenin (120 day-oral Collip units 3 times daily) and continues to maintain the satisfactory improvement.[1]

Case 4, injection and oral therapy. R.F., age 45, was an example of radiation castration for menometrorrhagia due to uterine fibroids 3 years previously. Symptoms complained of were vertigo, hot flushes, headaches, dyspareunia and vulvo-vaginitis. A specimen for a vaginal biopsy taken on November 3, 1937 (fig. 3, case IV, A) showed a low vaginal epithelium. The patient was placed on intramuscular injections of dihydroxyestrin benzoate (progynon-B, 4,000 R.U. 3 or 4 times a week) for 6 weeks. At the end of that period there was marked improvement in all symptoms and a specimen for a vaginal biopsy made on December 31, 1937 (fig. 3, case IV, B) showed marked growth of the vaginal epithelium, keratinization and glycogen deposits in the squamous epithelium. Uterine bleeding lasting 2 weeks necessitated an interruption in the therapy, and the patient was later placed on oral estrogenic medication (progynon-D-H tablets, 200 U 3 times daily) which were later reduced to 50 U 3 times daily. Estrogenic therapy was finally discontinued for control purposes and because of the presence of hypertension, sedative therapy was instituted for a period of 3 weeks, following this all therapy was discontinued by the patient for 2 months. This was followed by a gradual return of all symptoms previously complained of with the addition of arthritis and neuritis, the complaints relating to the vulvo-vaginal area, however, were not prominent. A specimen for a vaginal biopsy made on January 7, 1939 (fig. 3, case IV, C) showed only partial regression in the vaginal epithelium. She was then placed on progynon-D-H tablets 600 oral units 3 times daily with subsequent improvement in all symptoms. The biopsies made on February 29, 1939 (fig. 3, case IV, D) and March 23, 1939 showed a growth of the vaginal epithelium. The estrogenic therapy is now being gradually reduced to the minimum of 50 oral units 3 times daily and constitutes a satisfactory maintenance dose.

Case 5, oral therapy. Mrs. D.S., age 48, a white female showed a tendency to acromegalic facies and shape of hands. Spontaneous menopause occurred 8 years previously. Symptoms complained of were pruritus vulvae and discharge as well as headaches, vertigo and periodic edema of the face and hands. Radiological examination of the sella turcica pointed towards the possibility of a pituitary neoplasm. Eye ground and neurological examinations and repeated radiological studies did not confirm this tentative diagnosis. A specimen for a vaginal biopsy was taken on March 28, 1939 (fig. 4, case V, A) and showed low vaginal epithelium of senile type with leucocytic infiltration of epithelial and sub-epithelial layers. The patient was placed on estrobene (diethylstilbestrol) 1 mg. 3 times daily for 4 weeks with a resulting improvement in the local as well as general symptoms. At the end of this period, another specimen for a biopsy was taken on April 27, 1939 (fig. 4, case V, B) and showed marked growth of the vaginal epithelium with glycogen deposits in epithelial

[1] The contrast between this moderate maintenance dose and the large doses used in beginning the treatment suggests that emmenin is not very efficiently utilized when large amounts are given, possibly because its high solubility permits rapid passage through the body.

cells and disappearance of leucocytic infiltration. Following this, the dosage was reduced to 1 mg. once daily and on May 23, 1939 the patient experienced a profuse uterine bleeding lasting one day. The medication has been temporarily discontinued, and another form of estrogenic therapy will be administered orally in the near future.

SUMMARY

Of a series of 37 patients presenting symptoms of senile vulvo-vaginitis and treated with estrogens, 21 were treated by topical inunction with estrogenic ointment and 16 were treated by oral estrogenic substances.

Topical application of estrogens in a suitable base (lanolin) especially in patients who have none or only slight general menopausal symptoms, proved to be of marked benefit in alleviating the symptoms of senile vulvo-vaginitis and produced pronounced changes in the vaginal histology.

Patients presenting inflammatory changes superimposed on an atrophic vaginal mucosa responded rapidly to local treatment. This was evidenced by the alleviation of the symptoms and the changes both in the histological picture and in the vaginal smear.

Evidence that systemic absorption takes place in the course of inunction was manifested by slight to moderate improvement in the vaso-motor symptoms of the menopause and a recurrence of uterine bleeding in one patient.

Oral administration of estrogens proved also to be of great value, producing similar beneficial effects. This form of therapy is indicated in patients presenting marked vaso-motor disturbances in addition to the symptoms of senile vulvo-vaginitis. In many instances oral medication was substituted for intramuscular medication, because of the reappearance of uterine bleeding in patients treated by injection. Uterine bleeding, however, occurred in the 2 patients treated orally with diethylstilbestrol. This would seem to indicate unusual oral activity of the substance. Such bleeding did not occur in patients receiving progynon-D-H or emmenin.

Of the 21 patients treated by inunction, 6 obtained partial relief, while 15 showed marked improvement. In the former a change to injection therapy was made without marked improvement except in one instance. It should, however, be pointed out that there were complicating factors present such as diabetes, eczema, extensive leukoplakia and hypertrophic pathological changes of the vulva. Of the 16 patients treated with oral medication, one failed to respond.

Better cooperation on the part of the patient was obtained when the treatment was given orally.

Appreciation is expressed to Dr. Zelda I. Marks for her cooperation in the treatment of the patients, to Miss Ann Cooper for the microphotography and to Miss Bessie Zirin for technical assistance.

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THE EFFECT OF LARGE DOSES OF TESTOSTERONE PROPIONATE (ORETON) ON THE FEMALE GENITAL TRACT OF THE VERY YOUNG RAT. PRODUCTION OF OVARIAN CYSTS

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EXTENSIVE studies of the effects of the male sex hormones on female experimental animals have been made, which report changes in ovaries, uterus, female prostate, clitoris, adrenals, pituitary and estrous cycle. The results are not usually comparable because different androgenic compounds were used. However, the effect of really large doses of male sex hormone did not seem to us to be definitely settled. What dosage shall be considered large is obviously an open question but it seemed to be of some interest to study prolonged treatments with doses generally higher than those reported.

The experiments reported in this paper were performed on Wistar strain rats under constant conditions of food, light and temperature. Subcutaneous injections of testosterone propionate[1] were started on the first or second day of life. Litter-mate controls were used throughout the experiment. In one series, the litter-mate controls received nothing; in the second, not yet completed, they received equivalent amounts of sesame oil, the solvent for the testosterone propionate. Three injections of 1 mg. each were given weekly. In some of the experiments 0.5 mg. doses were given during the first 2 weeks and the full dosage thereafter. Some animals were killed after doses ranging from 51 to 182 mg., while others are being studied for late effects after treatment. Nine of the animals killed received a total dosage of 100 mg. and above, and 6 had between 51 and 100 mg.

EXPERIMENTAL RESULTS

Opening of the vagina occurred in 30 of 43 treated rats, while in 13 the vagina failed to open. The vagina opened in all the controls. In the treated animals opening of the vagina occurred between the 14th and the 75th day of life (average 23 days), in the controls, between the 25th and the 48th day (average 31 days). Considering that the time of vaginal opening varies considerably in different litters, but is quite constant for the members of the same litter, averages such as those given here may be of doubtful value.

[1] We are indebted to Dr. Max Gilbert of Schering Corporation, Bloomfield, N. J., for our supply of testosterone propionate (oreton) used in these experiments.

Comparing litter-mates of 15 litters, and considering differences of one to two days as normal variation, we found vaginal opening to occur at the same time in the controls and treated animals in 4 litters. In 6 litters vaginal opening of the treated animals was delayed and in 5 litters it occurred earlier in the treated animals. In one litter, the vagina of one of the treated animals opened considerably later than that of the control, while in a second treated litter mate it opened considerably earlier.

Effect on Estrus

Vaginal smears were made daily after the vagina opened. In most cases there were occasional positive smears (cornified epithelial cells) at different stages of treatment which were apparently independent of the total dosage of testosterone propionate. These occasional positive smears were not preceded by a pre-estrous smear (nucleated epithelial cells) nor followed by a post-estrous smear, which would have indicated a regular building-up of the vaginal mucosa. Isolated pre-estrous smears with nucleated epithelia only were obtained more frequently than estrous smears. Again, this occurred without any regular cyclic rhythm and failed to develop cornification. While a true cycle was absent in 30 animals, there were some exceptions: 3 treated rats had one complete cycle each, after a total dosage of testosterone propionate of 27.5, 20.5 and 4.0 mg. respectively. They never showed a cycle again. *Rat 146B* presented 4 complete estrous cycles of 6 to 8 days duration, went into diestrus and, after a month, again started cycles. After 3 cycles of 6 to 11 days duration, it again went into diestrus and only occasionally showed nucleated epithelia, never again showing a true cycle or cornification. At the time the last cycle was observed, this rat had received a total dosage of 64 mg. of testosterone propionate. The controls all showed normal estrous cycles.

Fertility

Mating was attempted 14 times in 6 treated rats. No pregnancy occurred. The fertility of the males used in these mating experiments was checked each time by mating with normal females. None of the bucks was sterile.

Changes in Clitoris

The clitoris was considerably enlarged in all treated animals. It was difficult to decide at what dosage this occurred because the enlargement progressed continuously. After as little as 1 or 2 weeks of treatment, when the animals had received only 3 to 6 mg., the immature clitoris was larger than that of the control. The exaggerated picture of a penis-like clitoris with purple, bulbous glans was present after a total dosage of 20 to 30 mg., at an age of 2 to 3 months. The preputial glands on pressure, produced ample yellow, creamy material.

Development of Ovarian Cysts

In the course of treatment a very conspicuous change occurred. A number of animals developed palpable abdominal tumors. In some, with a

total dosage around 40 mg., large uneven lobulated masses were palpable in the lateral abdominal region. Tumors were present on one or both sides of the abdomen. The tumors usually were movable. Their size was not related to the total dose of hormone, since the tumors in animals having received 45 mg. were sometimes considerably larger than in those which had received more than 100 mg. Even in members of the same litter, treated in identical fashion, one animal may develop tumors while another fails to do so. These facts were confirmed by the autopsy findings and will be discussed more

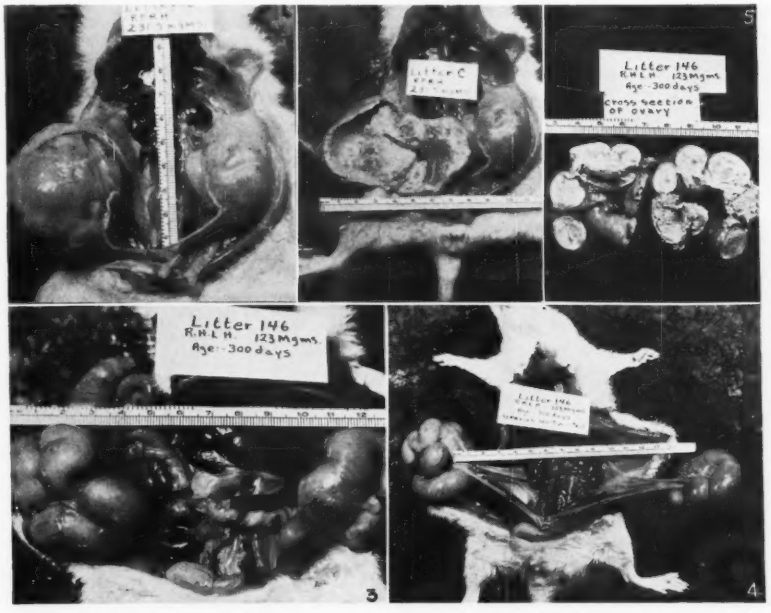

Fig. 1. LARGE OVARIAN TUMORS. Peritoneal adhesions. The right tumor gives the impression of being monolocular. Fig. 2. SAME ANIMAL AS IN FIG. 1. Right tumor opened, caseous contents removed. Note low septa. Fig. 3. LARGE MARKEDLY MULTILOCULAR TUMORS OF BOTH OVARIES. Note twisting of uterus and vessels. Fig. 4. SAME ANIMAL AS IN FIG 3. Uteri untwisted. Note length of uterine horns. Fig. 5. CROSS SECTION OF RIGHT OVARY OF THE SAME CASE. Note caseous contents.

fully later. Tumors of the kind described were present in 25 of 37 treated animals. The total dosage of hormone administered ranged between 40 and 180 mg.

Postmortem Findings

Fifteen treated animals and 6 untreated controls were killed at various stages of the experiment. Several animals that died during the experiment were dissected, but the findings are not included in this report due to the fact that decomposition prevented reliable organ weights and microscopic studies. In 2 animals, the tumors were removed operatively. The remainder

of the animals are still alive and are being observed for prolonged effects as well as changes after treatment.

The animals were anesthetized with chloroform, the thorax quickly opened, and the animals killed by the double action of bilateral pneumothorax and bleeding by opening the heart. The organs were removed, dis-

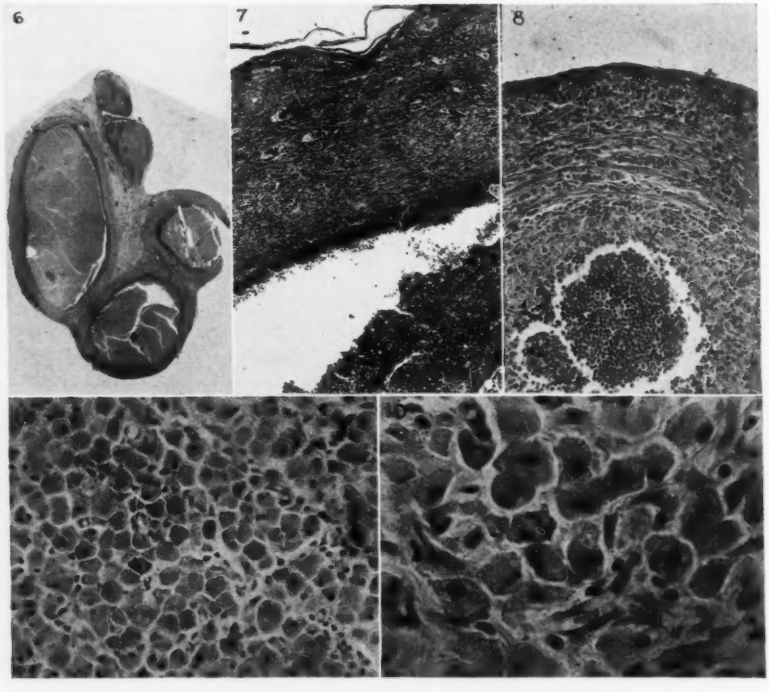

Fig. 6. *Rat 179 LFLH.* TOTAL DOSAGE 105 mg. Cross section of small ovarian tumor. Stained hematoxylin-eosin. 3×. Note leucocytic layer and central necrosis. Fig. 7. *Rat 146 b.* TOTAL DOSAGE 126 mg. Cyst wall. Caseous contents of cysts to the right. Leucocytic layer, fibrous tissue. No lutein cells visible. Pictures like this seen in our first cases, could not be explained as to their genesis. Stained Van Gieson. 35×. Fig. 8. *Rat 104 RFRH.* TOTAL DOSAGE 182 mg. Smaller cyst. Note well preserved lutein cell layer and fibrous capsule. Compare with figure 7, where destruction of lutein cells is complete. Stained hematoxylin-eosin. 110×. Fig. 9. *Rat 99 LF.* TOTAL DOSAGE 55 mg. Lutein cells in small ovarian tumor without cystic degenerative changes. Stained hematoxylin-eosin. 225×. Fig. 10. SAME AS FIGURE 9. 480×.

sected free from connective tissue, and weighed. At first an analytical balance was used but we found a torsion balance more convenient, the time of actual weighing being greatly reduced. This not only saves time but also reduces the error due to desiccation. The organs were then fixed in Zenker-formol, embedded in paraffin and sections of 5 to 7 micra made and stained accordingly.

Changes in Ovaries

Changes in the ovaries were found in all of the 15 animals killed, as well as in those on which autopsy was performed after spontaneous death, and in 2 animals on which operation had been performed. The changes may be studied in two groups: (*a*) enlargement, (*b*) atrophy.

(*A*) *Enlargement.* Ten of the 15 animals killed had enlarged ovaries. The size varied from 7 mm. to 50 mm. (longest diameter). In this series, the ovaries of the 2 animals in which ovariectomy was performed are included, bringing the total number to 12. The size of the ovarian tumor, as previously suspected, was not related to the total dosage of testosterone propionate received. The tumor weight ranged between 948 and 62,600 mg. (both ovaries weighed together). In two of the litters, (146, 179) some of the treated animals had tumors while their litter mates treated with the same dosage of hormone showed atrophic ovaries. In one of the animals (104 *l.f.*) the right ovary was transformed into a tumor weighing 27,000 mg. while the left ovary was atrophic (weight 10.4 mg.).

The tumors appeared externally as multilocular formations, but in some cases they seemed unilocular. On opening, the tumors nearly always showed septa, sometimes complete, sometimes incomplete. The color of the small tumors was yellow; the larger, especially those with kinked blood vessels (see below) were dark red, due to congestion.

The contents of the cystic tumors was in all cases a thick, yellowish, caseous material. In one instance only was it more liquid and putrid. Smears made of the caseous content in several cases failed to reveal anything but amorphous detritus. In 3 instances bacteriological cultures of the caseous content were made on agar and in broth. In one there was no growth; in the second, bacillus coli and in the third, bacillus proteus. The inner surface of the cystic tumors after removal of the caseous content sometimes appeared dull and granular.

The uterus, together with the uterine vessels, was twisted in 3 of the animals. The resulting picture resembled pedunculated, twisted ovarian cysts in human pathology. It is needless to emphasize that the twisted peduncle in the experimental rats was the uterine horn. One of the striking effects of this twisting was the congestion of the organs supplied by the uterine vessels. This congestion was even more marked where the large ovarian tumor had 'changed sides,' the right ovary lying in the left abdomen covering the left ovary, or vice versa. In these cases, there was obviously a kink of the peduncle besides the twisting.

(*B*) *Atrophy.* In cases in which the ovaries were found atrophic, the fat along the uterine horn and in the ovarian region was extremely well developed, so much so that only with some difficulty was it possible to find the small white atrophied ovary and never was it possible to separate this organ from the oviduct.

In the tumor cases, the uterine horns were usually elongated, measuring

up to 11 cm. in length. Generally they were pale and thin, but in 3 animals they were flabby and in one markedly distended and yellowish in color. The vagina was transformed into a solid thin cord in three animals of one litter. In the others there were no macroscopic vaginal changes. The preputial glands were markedly enlarged in all cases.

The appearance of the clitoris was described above. In several cases the course of the urethra was followed by entering a probe through the bladder. This was not done in all cases for fear of damaging the epithelium and interfering with proper histological examination. In many instances the clitoris was hypo-spadic and the urethral opening was located here. In others the urethral opening was found in normal position.

The anterior wall of the upper urethra was reddish-brown in color in all the animals. This has been ascribed to the presence of prostatic glands. We shall discuss the significance of this finding in a later publication. It is enough to state here that presence or absence of prostatic tissue was in no way to be deduced from the finding of this brown-colored anterior urethral wall.

Histology

Histological examinations of the ovaries, uterus and vagina only are recorded in this paper. Histological findings of the other endocrine organs will be reported separately later.

The walls of the large ovarian, cystic tumors showed no specific tissue. They were composed of layers of connective tissue loosely textured and rich in fibroblastic cells. In those sections in which they were preserved, the contents of the cysts were amorphous, crumbly masses. Between the caseous mass and the cyst wall there was generally a layer of leucocytes which had invaded the inner layers of the cyst wall. The smaller cysts usually showed the same structure, but in a number of cases the inner layer of the cyst wall carried large round or polygonal cells with comparatively small vesicular nuclei often eccentrically situated. The cytoplasm was granular and in many instances vacuolated. It was difficult to classify these cells because they were quite degenerated; probably a result of the leucocytic infiltration. However, in other specimens we found layers of such cells throughout the cyst wall where the more peripherally located strands were well-preserved. Frozen sections of ovarian cysts fixed in 10% formalin stained with Sudan III revealed small fat droplets in these lutein cells. The study of some of the smaller ovarian tumors offered further identification of these cells. We found the same type of cell in large nodular groups, which were easily recognizable as remnants of corpora lutea, and into some of these grew slender strands of connective tissue.

It seemed that extensive luteinization was the process involved. In smaller tumors there was evidence of regression of the corpus luteum as seen physiologically; namely, an ingrowth of connective tissue subdividing the lutein cell layers. In these cases the groups of more or less intact lutein cells were not walled off by a capsule. The next stage showed the corpora lutea with partial necrosis and leucocytic infiltration.

The connective tissue walls of the larger cystic tumors were apparently derived from the external theca. Large tumor-like formations seemed to develop only after luteinization had taken place in the regular way within a follicle. Some of the smaller ovaries give the impression that luteinization had taken place within the interstitial tissue but this could not be conclusively proved. We feel inclined to explain the finding of the epithelial lining in parts of the smaller cysts as a remnant of granulosa cells, though we cannot explain why they had escaped luteinization and thus failed to be transformed into granulosa lutein cells. In 2 cases microscopic examination revealed small cysts with cuboidal epithelial lining and without any trace of luteinization, obviously follicle cysts.

The microscopic findings in the ovaries described as atrophy can be summed up more briefly. Mostly connective tissue only was found and that adherent to the tubules of the oviduct.

In 3 ovaries of which serial sections were made, immature ova were found and in one of these there were mature follicles. In another the sections revealed immature ova, several maturing follicles and extensive luteinization though the ovaries macroscopically had impressed us as being markedly atrophic.

Histology of the uterine mucosa of the rat is a rather difficult subject. It should be emphasized that W. M. Allen's study (1) of the cyclic changes of the uterine mucosa has been extensively used to check our findings and to attempt to correlate them with ovarian processes. However, the uterine mucosa in our experimental rats rarely presented clear-cut pictures that were in exact correlation to single phases of the estrous and pseudopregnant cycle of the normal rat. This was to be expected since the animals were under the influence of large doses of male sex hormone which could act either directly on the uterus, or indirectly by producing ovarian changes.

In 3 cases the uterine lumen and the glands were distended with a marked leucocytic invasion in both epithelium and stroma. This is considered by Allen to be characteristic of estrus. In one only of these 3 was the epithelium vacuolized. Mitoses were absent in all 3. In 2 of these the vaginal epithelium was cornified. We may, therefore, consider these 2 and probably all 3 animals to have been in the estrous phase. Yet the picture is not in every respect typical. Two of the 3 presented a hillocky surface of the uterine mucosa in contrast to the smooth surface of the typical pre-estrous and estrous phases. Furthermore, these 2 presented a stroma with vesicular nuclei again contrasting with the fibroblast-like nuclei of the estrous phase. These 3 animals carried ovarian tumors. In none of the ovaries did we see any trace of follicles. However, as serial sections of ovarian tumors of this size were not practicable, the possibility remains that follicles could have been present somewhere.

One animal presented a uterine mucosa with high columnar epithelium, smooth surface, no distention of lumen, no leucocytic infiltration and no mitosis. This should be considered as a picture of an early pre-estrous phase, except that a basal membrane could not be found. The ovaries of this case

were atrophic but on serial section revealed maturing follicles. The other 4 uteri can be considered to have been in various stages of the corpus luteum phase corresponding to the picture of pseudo-pregnancy described by Allen. One of these animals had ovarian tumors, 3 had atrophic ovaries and in the latter no trace of luteinization was to be found.

DISCUSSION

Stimulation of luteinization in the rodent ovary has been described (2–7). Inhibition of ovarian growth following administration of testosterone propionate has been reported by McEuen and associates (8), and by Mazer and Mazer (9). The latter believe that small doses administered over a short period stimulate luteinization whereas prolonged treatment leads to atrophy. Freed, Greenhill and Soskin (7) describe a contrary bi-phasic action: small doses produce ovarian atrophy; large doses produce luteinization. Confusing and contradictory as these results may seem, it must be borne in mind that the results of various authors cannot on the whole be considered contradictory for the simple reason that the experimental conditions are frequently quite different. In our own series the finding of corpus luteum tumors is in accord with the observations of luteinization induced by testosterone propionate. Surprisingly, these corpora lutea persist under prolonged treatment and fail to undergo normal regression. Finally, however, the tissue turns necrotic and secondary invasion of intestinal bacteria (proteus, coli) occurs. What light the mechanism of the production of these rodent corpus luteum tumors is apt to throw on the genesis of corpus luteum tumors in man we do not know. However, the striking morphological similarity of the pictures we describe to those found in human pathology should be emphasized. The first detailed report on human corpus luteum cysts, published in 1895 by L. Fraenkel (10) should be consulted for comparison. The picture these tumor ovaries present is quite different from the ovarian atrophy described by numerous authors as well as from that seen in some of our experimental animals. Actually in these tumor ovaries the remaining ovarian tissue, too, may be considered atrophic.

Even though we have not seen any follicles in the ovaries transformed into tumors, there are findings in our animals which make the occasional presence of mature follicles probable. As has already been pointed out, suppression of estrus was not complete in a number of rats. The finding of occasional cycles during one treatment when the animal had received considerable amounts of testosterone propionate is not in accord with the data published by various workers (2, 9, 11, 12). These authors described full suppression of estrus in the rat under the influence of testosterone propionate. However, in none of their experiments was observation carried over a period so long as ours. Observation of *rat 146 B* would have given an impression of complete suppression of estrus had it not been continued for more than one month.

It has recently been pointed out (13) that vaginal smears do not always offer reliable information of the cyclic changes. It has been claimed that histo-

logic examination of the vagina should be substituted for the vaginal smear method under certain circumstances. Obviously, observation of animals over long periods cannot but be based on the smear method. Accordingly, this method is generally used and is found reliable provided nutritional and other conditions are kept constant (avitaminosis A, for example, enhances cornification). Apart from our observations of occasional cyclic changes, the histologic examination of the vagina revealed cornification in 2 animals while in 3 the uterine mucosa was highly suggestive of the estrous phase. We did not detect follicles in any of these ovaries but as, for obvious reasons, serial sections of the entire ovary could not be taken, we may assume that mature follicles were present. The same assumption should be made for the occasional cycles. Follicular maturation then is in disorder, no regular rhythms exist, yet occasionally a follicle matures producing cyclic changes in smear, vaginal wall and uterine mucosa.

The ovarian atrophy described was by no means complete. This was to be expected for Zuckerman (14), Mazer and Mazer (9) and others have seen a recovery of ovaries and of estrus after cessation of testosterone treatment. Accordingly, in 3 ovaries which appeared highly atrophic at autopsy serial sections showed ova which might have developed follicles after treatment had been stopped. In one of these 3, however, several maturing follicles were found to present a distinct theca, follicle cavity and cumulus oöphorus. It should be emphasized that this animal was killed 2 days after the last injection and had received a total dosage of 126 mg. It is apparent that even during prolonged treatment with large doses of testosterone propionate occasional ripening of follicles can occur.

McEuen, Selye and Collip (8) as well as Bottomley and Folley (15) failed to find any significant changes in pituitary size or weight following comparatively short periods of treatment with testosterone propionate. Mazer and Mazer (9), on the other hand, reported a marked decrease of pituitary weight after 104 days of treatment which was started at a very early age. This finding is confirmed by our own results. That the effect of sex hormones on the gonads is due to pituitary inhibition was emphasized as early as 1932, by Moore (16) and his collaborators. Administration of gonadotropic hormones or of pituitary implants prevented changes of the gonads following simultaneous administration of sex hormones. Castration changes of the pituitary are prevented by adequate doses of male or female sex hormone (17). The pituitary of animals treated with testosterone propionate has been proved to be less potent than normal pituitaries when implanted into immature animals (18). Injection of testosterone propionate or estrone, into the castrated parabiont prevented stimulation of the testes of the other, hypophysectomized twin. Stimulation would otherwise occur by output of gonadotropic hormone in the castrated, non-hypophysectomized parabiont (19). Salmon (20) has shown that the urinary output of gonadotropic hormone in a castrated woman can be greatly reduced by high doses of testosterone as well as of estrone. Similar though the action of female and male sex hormone on the pituitary may be their action yet is different in these

respects. Whereas estrone produces enlargement of the pituitary or even pituitary adenomas (21–24), testosterone propionate decreases pituitary weight. Furthermore, the hypopituitarism due to estrogen involves the growth hormone. This is not the case with testosterone propionate. Pending the results of microscopic examination of the pituitaries of our animals, which are to be published at a later date, we may assume that a decrease in weight of the pituitary indicates an inhibition of that organ. Thus, ovarian atrophy or rather maturation arrest may be ascribed to the pituitary inhibition, but this fails to explain the luteinization leading to the formation of ovarian tumors. This can be explained only by an isolated release of luteinizing hormone from the otherwise inhibited pituitary or by a direct action of testosterone on the ovary. Experiments on hypophysectomized rats should decide this question. Such experiments are now under way in our laboratory. In the hypophysectomized amphibian, Zwarenstein and Shapiro (15) have found ovarian stimulation by testosterone propionate.

We have started treating a series of rats with large doses of estrone under conditions identical to those described for testosterone propionate. This series is still under way and results are not yet available except for one animal which has received a total dose of 22,500 I.U. estrone (progynon) over a period of 7 months. Surprisingly enough this animal, too, had ovarian tumors of exactly the same appearance as had the testosterone treated rats. We do not wish to draw conclusions based on the findings from one animal. However, the result is suggestive of an indirect action on the ovaries possibly from release of luteinizing hormone by the pituitary.

SUMMARY

By administering large doses of testosterone propionate, beginning at the age of 1 day, ovarian tumors were produced in female rats. These tumors were shown to be lutein cysts. In some animals treated identically, atrophy of the ovaries without luteinization occurred.

The size of the lutein cysts was not directly related to the total dose of testosterone propionate nor to the duration of treatment.

The estrous cycle was not completely suppressed. Even after high total doses of testosterone propionate occasional cycles appeared in some animals with lutein cysts and with macroscopically atrophic ovaries. On one of the macroscopically atrophic ovaries, mature follicles were found. The uterus presented progestational pictures in most cases, but in 3 animals estrous changes were found. Occasional maturation of follicles can occur even after large doses of testosterone propionate. Since cycles occurred in animals bearing lutein tumors, the question may be raised as to how long the lutein tissue remains functional. This question cannot be answered at the present time.

The present conception is that administration of sex hormones affects the gonads by depression of the pituitary. Under this theory we would have to assume release of luteinizing pituitary hormone from the otherwise de-

pressed pituitary. However, a direct influence of testosterone on the female gonad has to be envisaged as another alternative explanation.

We gratefully acknowledge the technical assistance of Miss Miriam P. Blum.

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THE ACTION OF SMALL QUANTITIES OF PROGESTERONE

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Of the many effects that have in the past been attributed to the corpus luteum hormone (progesterone) there are only two that are used as a basis for rational biological determination of its strength: *a*), that of rendering the endometrium of spayed rats, mice and guinea pigs sensitive to the formation of deciduomata (1, 2, 3); and, *b*), the production of the pregravid phase (the decidual or secretory phase) in the endometrium of rabbits.

The latter test, which was first described by Corner and Allen (4), later modified by Clauberg (5), and which has since been described many times in the literature (6, 7, 8) is the most accurate and the one most used. It is usually carried out—as, for instance at the biological laboratory at Løvens kemiske Fabrik, Copenhagen—by Clauberg's method. The hormone is injected into immature rabbits that have previously been treated with estrin and the endometrium is subsequently examined histologically. Determination of potency by this method shows that about 0.75 mg. of progesterone must be used to produce the entire secretory phase. The international progesterone standard contains 1 I.U. in 1 mg., or a little more than 1 rabbit unit.

The hormonal function shown by these tests does not seem to be the same as that which produces relaxation of the guinea pig pubic symphysis (9, 10), nor is it identical with that which acts antagonistically to oxytocin (11, 12). Knaus has proposed the antagonistic action to oxytocin in vitro as a basis for standardization.

Although the commonly used Clauberg test is reliable and fairly accurate it has certain disadvantages. It is elaborate and expensive, and it is only possible to demonstrate relatively large quantities of progesterone by means of it. This, perhaps, is the reason why it has not been possible to demonstrate the presence of progesterone in the urine or in the blood. Venning and Browne (13), who evolved a method of extracting sodium pregnandiol glucuronate from urine, investigated the content of this substance, which closely approaches progesterone chemically, in the urine of pregnant and non-pregnant women. They are of the opinion that progesterone is not excreted as such in the urine but is hydrogenated to pregnandiol, which is then excreted. Apart from its possible presence in the placenta (14, 15) progesterone is only demonstrable with certainty in corpora lutea. There is therefore good reason to attempt to find a new, easier, cheaper and more sensitive, test for progesterone.

The following is an account of experiments made to evolve such a quantitative test and, even though success has not as yet been attained, unusual results have appeared which warrant description.

The motive for these investigations appeared during work on gonado-

tropic hormone analysis of urine in accordance with Thomsen and Pedersen-Bjergaard's method as published in 1936 (16). It appeared that even though the well-known large uterus, filled with liquid, was most frequently seen in those rats that showed positive reactions and were autopsied 72 hours after the first injection, an empty but hypertrophied uterus was found in some animals. The weight of these uteri was at about the lower limit for a 'positive reaction'; the ovaries, however, showed action of the gonadotropic hormone distinctly. Microscopically, a slightly distended lumen, limited by a hyper-

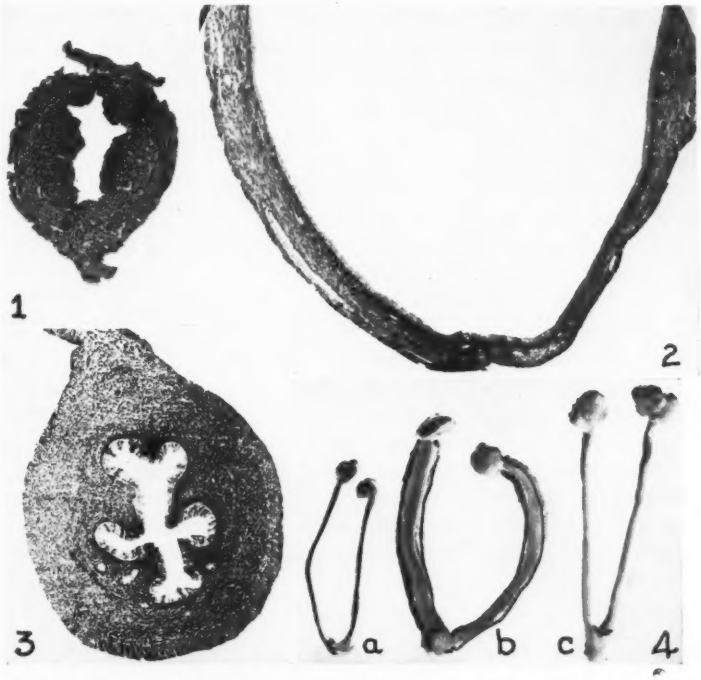


Fig. 1. Cross section of uterus from an untreated immature rat. Fig. 2. Cross section of the dilated type of uterus. Fig. 3. Cross section of the contracted type of uterus. Fig. 4. Uterus and ovaries from a), an untreated immature rat, b), a rat showing the dilated type of uterus, c), a rat showing the contracted type of uterus.

trophied but thin wall (because of distention) with cuboidal epithelial cells, was seen in the first form; a star-shaped or rosette-shaped lumen, surrounded by a very greatly hypertrophied wall, and with a high cylindrical epithelium was seen in the second.

These two forms (fig. 1 to 4) have been described previously. Even in their first work on the action of the anterior pituitary hormone on immature rats and mice Smith and Engle (17) say "that in estrus the uterus passes from a stage in which it is distended with secretion to a hypertrophic, but non-dilated stage. These changes which may take place within 24 hours are effected by evacuation of the uterine secretion."

Hamburger (18) described these two forms of estrual reaction in the uteri of mice in 1933, the animals being killed in this case from 96 to 100 hours after the first injection. He found that extracts of human-castrate urine, as well as that from the urine of pregnant women during the last months of pregnancy, produced the contracted uterus, and urine extract from women at the beginning of pregnancy, the dilated uterus. The dilated uterus could also be found after the injection of human-castrate urine, though not as a constant phenomenon, when the animals were autopsied 72 hours after the first injection. Hamburger also mentions that he did not succeed in producing a distinct picture of dilated uterus from injecting estrogenic hormone (Ovex Leo) in doses of from 5 to 50 M.U. He was not able to arrive to any definite conclusion from his material as to the conditions necessary for the production of the two types, but he found that there is a connection between the production of the contracted type and the luteinizing action of the gonadotropic extracts and, after having described the dilated uterus with the thin and transparent wall, says that "the latter change does not appear, however, when corpora lutea develop in the ovaries (effect of luteinizing hormone). In such cases the uterus is most found to be in a phase corresponding to the progestational phase in the rabbit."

We found that the appearance of the contracted type in our rats that were used for gonadotropic hormone assays and autopsied 72 hours after the first injection was dependent on whether developed corpora lutea were found in the ovaries, as we always found corpora lutea (either microscopically or macroscopically) in the contracted type, and never in the dilated. As is well known the uterine reactions after the injection of gonadotropic hormone are secondary, being caused by the demobilized ovarian hormones.

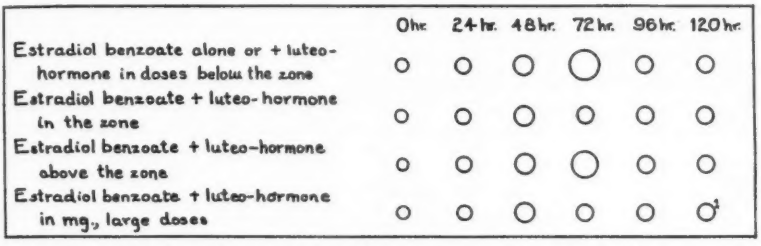
We then investigated the action of estradiol benzoate on the uterus of the rat. The results can be seen in table 1. The experimental animals were 21-day-old rats of our own inbred constant M-C-breed. Their weight was about 30 gm. They were given a single subcutaneous injection of 0.5 cc. of olive oil containing 2γ of estradiol benzoate.

TABLE 1

Killed after	No. of rats	% dilation	% contraction	Average all	Weight of uterus	
					dilated	contracted
hr.						
48	20	100	0	142.6	142.6	—
60	21	100	0	141.4	141.4	—
72	21	100	0	153.6	153.6	—
84	20	60	40	137.1	170.0	87.6
96	20	0	100	82.5	—	82.5

It can be seen from table 1 that dilated uteri were found in all the animals when they were autopsied after 48, 60 and 72 hours. After 84 hours the uteri of 40% of the animals were evacuated, and after 96 hours all of the uteri were of the contracted type. We have, in the course of time, experimented so often with the action of estradiol benzoate alone (with other doses and with animals of other ages than those shown in

table 1) that we dare say that the action after 72 hours is quite constantly that shown in the tests referred to, that is great dilation of the uterus, which is filled with fluid. We might therefore consider that the contraction that we have observed after 72 hours is due to the progesterone from the corpora lutea produced in the ovaries of the experimental animals by gonadotropic hormone injected.


0 hr.
24 hr.
48 hr.
72 hr.
96 hr.
120 hr.
Estradiol benzoate alone or + luteo-hormone in doses below the zone
Estradiol benzoate + luteo-hormone in the zone
Estradiol benzoate + luteo-hormone above the zone
Estradiol benzoate + luteo-hormone in mg., large doses
1

[1] This reaction or larger dilation if estradiol benzoate can be active at this period.

Fig. 5

In the next series of experiments we tried to imitate this by giving a number of rats varying doses of progesterone (Lutex Leo, prepared from the ovaries of pigs) combined with a definite amount of estradiol benzoate (2γ). The dose of hormone was administered dissolved in 0.5 cc. of olive oil, subcutaneously, in one injection. Autopsy was performed 72 hours after the injection. The experimental animals were rats from 28 to 30 days old.

TABLE 2

Weight of rat	Dose progesterone	Weight of uterus	Contraction	Dilation	Weight of rat	Dose progesterone	Weight of uterus	Contraction	Dilation
gm.	RB.U	mg.			gm.	RB.U	mg.		
66	1.0	265		+	46	0.20	108	+	
47	1.0	249		+	52	0.20	98	+	
48	1.0	195		+	58	0.20	123	+	
45	1.0	223		+	54	0.13	101	+	
39	0.5	264		+	53	0.13	112	+	
42	0.5	205		+	47	0.13	78	+	
76	0.5	202		+	55	0.13	134		+
54	0.5	242		+	57	0.13	123		+
50	0.33	222		+	48	0.10	99		+
60	0.33	250		+	48	0.10	85		+
63	0.33	148		+	53	0.10	146		+
57	0.33	115	+		46	0.10	114		+
56	0.33	118	+		44	0.067	100		+
55	0.25	101	+		50	0.067	197		+
55	0.25	104	+		44	0.067	165		+
60	0.25	103	+		57	0	290		+
49	0.25	176	+		62	0	252		+

As can be seen from table 2 this produced an extraordinary result. The contracted uterus appeared in a definite zone of varying quantities of progesterone (Lutex), the dilated type of uterus being found not only over, but also under the zone. The zone of action was found, as can be seen, to be between 0.3 and 0.13 RB.U.

The next experiment, which was carried out in the same way but with

another Lutex preparation, produced exactly the same results (table 3), except that the zone of action was in this case between 0.06 and 0.02 RB.U., inclusive.

From this it can be seen—and it is not extraordinary that it should be so—that some variation occurs when the doses reach the limits, some of the animals showing one and some the other type of uterus.

The material on which the data of table 2 are based is, moreover, pure, for we found that the contracted uterus appeared now and then with doses

TABLE 3

Weight of rat	Dose progesterone	Weight of uterus	Contraction	Dilation	Weight of rat	Dose progesterone	Weight of uterus	Contraction	Dilation
gm.	RB.U	mg.			gm.	RB.U	mg.		
26	1.0	154		+	36	0.05	85	+	
20	1.0	90		+	34	0.05	187		+
22	1.0	116		+	33	0.05	85	+	
25	0.5	160		+	26	0.05	88	+	
28	0.5	110		+	34	0.03	79	+	
26	0.5	137		+	37	0.03	91	+	
22	0.5	100		+	40	0.03	84	+	
25	0.5	150		+	31	0.02	90	+	
16	0.33	123		+	43	0.02	94	+	
39	0.33	206		+	42	0.02	92	+	
17	0.25	124		+	42	0.02	85	+	
26	0.25	168		+	42	0.02	76	+	
27	0.25	150		+	42	0.01	88	+	
30	0.25	131		+	25	0.01	226		+
30	0.25	142		+	25	0.01	127		+
21	0.20	151		+	22	0.01	153		+
17	0.20	112		+	26	0.01	208		+
50	0.20	207		+	26	0.005	225		+
24	0.13	146		+	24	0.005	225		+
24	0.13	190		+	20	0.005	145		+
24	0.13	91		+	30	0.005	132		+
24	0.13	75		+	30	0.005	245		+
19	0.13	143		+	20	0	87		+
21	0.10	91		+	20	0	107		+
34	0.10	76	+		25	0	93		+
28	0.10	149		+	22	0	104		+
34	0.10	242		+	22	0	150		+
18	0.067	60	+		22	0	175		+
34	0.067	84	+		34	0	139		+
35	0.067	78	+		32	0	150		+

that were above, as well as those that were below, the distinct zone of contraction.

On closer inspection all the rats that showed 'defective reaction' proved to have well developed corpora lutea in their ovaries. This, as Hohlweg (19) showed at about the same time, is due to the fact that the estradiol benzoate injected into rats of this age mobilizes the luteinizing hormone from the animal's pituitary lobe. This will occur in about 30% of the cases when 2γ of estradiol benzoate is injected. On the contrary, estradiol benzoate produces no luteinization of the ovaries of 23-day-old rats. Hohlweg and Chamorro (20) showed later that hypophysectomy, performed 2 days after the injection, prevents the development of corpora lutea, but that when it is performed 4 days after the injection luteinization occurs just as in normal non-hypophysectomized animals. The mobilization of the luteinizing hormone

thus occurs, or at any rate begins, at the point of time between 48 and 96 hours after the injection. Hohlweg and Chamorro observed fully developed corpora lutea at the expiration of 5 days; we have found them as early as at the end of 3 days (our rats were somewhat larger than Hohlweg and Chamorro's). The data on these rats were, of course, excluded and after the first series of experiments we employed only 21-day-old rats.

Table 4 shows 6 of the animals excluded from the first series of experiments. Rats 4949 and 5468, which were given doses over and under the zone, respectively, had contracted uteri, as was not the case with the other rats on these doses; moreover, corpora lutea were found to be macroscopically and microscopically visible in their ovaries. Rats 5127, 5472 and 5473, like the others on the dose within the zone, showed contracted uteri, but they had large corpora in their ovaries, for which reason they were excluded. The ovaries of all the animals included in tables 2 and 3 were examined microscopically, but no corpora lutea were found.

TABLE 4

Weight	Dose	No.	Weight of uterus	Contraction	
gm.	RB.U.		mg.		
65	1.0	4949	108	+	The other rats on this dose were dilated.
57	0.200	5127	156	+	The other rats on this dose were contracted
76	0.200	5472	118	+	
77	0.200	5473	131	+	
81	0.067	5468	92	+	The other rats on this dose were dilated
69	0.067	5469	117	+	

It was also found that the mixture, progesterone-estradiol benzoate, became weaker if it was allowed to stand at ordinary room temperature, which might give rise to irregularities. Therefore when we did not use the whole of the freshly-prepared solution we kept the remainder of the liquid at a temperature of −6° C.

As has been mentioned above, we considered that the contraction of the uterus was the result of the action of progesterone on the uterus that had been already prepared by estradiol benzoate and we were therefore very surprised that this hormone became apparently inactive when the dose was increased above a certain point (the upper limit of the zone of activity), though it was, of course, to be expected that a lower limit existed. We thought that it might be possible that substances that restricted contraction might exist in the organic preparation, Lutex Leo, and that when the dose was increased above a certain limit it obtained better conditions for its activity than the luteo-hormone that produces contraction. We therefore decided to make our next experiments with a purer substance, a quasi-crystalline substance prepared synthetically from stigmasterin (progesterone Leo II). The result appears in table 5.

It can be seen that a distinct zone for this preparation is found here also, lying between 0.12 and 0.06 RB.U. A very similar action is obtained from

another crystalline substance, also prepared from stigmasterin (progesterone Leo I), the zone of contraction being here found to be with about 0.1 Rb.U. Thus the possibility was disproved that the organic preparations contained special restrictive substances, originating from the ovaries of swine, that were the cause of the upper limit of action. Another explanation had, therefore, to be sought, and this we think we have been successful in finding.

It can be seen from table 1 that the uterus is found to be dilated 72 hours after the injection of estradiol benzoate alone, spontaneous contraction not setting in until between the 84th and the 96th hour. Contraction is found to occur after 72 hours when a certain quantity of progesterone (within the zonal doses) is added, and it is probable that the question is one of acceleration of the spontaneous contraction (a pharmaco-dynamic action of the

TABLE 5. SYNTHETIC PROGESTERONE II. ALL RATS ARE 21 DAYS OLD

Weight of rat	Dose progesterone	Weight of uterus	Contraction	Dilation	Weight of rat	Dose progesterone	Weight of uterus	Contraction	Dilation
gm.	Rb.U.	mg.			gm.	Rb.U.	mg.		
33	1.0	154		+	28	0.13	116		+
33	1.0	171		+	35	0.13	90	+	
32	1.0	164		+	34	0.13	79	+	
36	1.0	134		+	36	0.13	117	+	
33	1.0	81		+	35	0.13	86	+	
50	1.0	180		+	30	0.06	85	+	
44	1.0	173		+	36	0.06	74	+	
28	0.5	148		+	34	0.06	72	+	
30	0.5	173		+	36	0.06	90	+	
32	0.5	110		+	27	0.06	214		+
28	0.5	97		+	20	0.06	213		+
26	0.5	196		+	32	0.06	129		+
28	0.25	160		+	44	0.06	163		+
28	0.25	160		+	32	0.03	117		+
28	0.25	216		+	34	0.03	92		+
34	0.25	250		+	31	0.03	109		+
30	0.25	264		+					

hormone injected). Doses below the lowest zonal limit must be considered to be too small to give any recognizable accelerated action. Doses over the highest zonal limit must be considered as acting in the same direction as those that are within the zone, but more powerfully and, therefore, with an action that can be recognized earlier, perhaps so early that no dilatation at all occurs during the first 48 hours. When the progesterone is thus consumed, that is when its action has ceased, the estradiol benzoate, the action of which is of longer duration, causes the usual dilatation, which is distinctly apparent and perhaps at its height when the animal is killed after 72 hours. As a corollary to this line of thought it is possible that very large doses will give a contraction that is still present after 72 hours though we have not as yet had the opportunity of proving this. Thus we obtain an explanation why the large doses of lueto-hormone (above the zone) apparently give the same result as the fairly small ones (below the zone). The various possibilities are shown in figure 5.

In all its essentials we have realized the above-mentioned principle, as we have examined the condition of the uteri of a series of rats autopsied 24, 48, 72, 96 and 120 hours after the injection of doses within and above the

zone. The result appears in table 6. It can be seen that the two doses above the zone (see table 8) produced dilatation after 72 hours, first (to the greatest degree for the higher dose) having produced contraction or, rather, restricted dilation of the already hypertrophied uterus (the uteri of the control animals weighed from 15 to 20 mg.).

TABLE 6

	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.
Within the zone 1/50 I. U.	56 mg.	111 mg.	65 mg.	63 mg.	40 mg.
Above the zone 1/10 I.U.	55 mg.	111 m.g	132 mg.	46 mg.	27 mg.
Above the zone 2 I.U.	49 mg.	60 mg.	112 mg.	65 mg.	58 mg.

As the present work was nearing completion a short account (16) appeared which contained the observation that the distention of the uterus by fluid, otherwise produced by estradiol, could be prevented by the simul-

TABLE 7. 21-DAY-OLD RATS, GIVEN 2γ ESTRADIOL BENZOATE+PROGESTERONE (PROLUTON SCHERING) SUBCUTANEOUSLY IN VARYING DOSES IN 0.5 CC. OLIVE OIL. THE ZONE OF ACTION IS FROM 0.06 TO RB.U.

Dose I.U.	Uterus, macroscopic			Weight of uterus in mg.		
	Total contract.	Total dilat.	% Contract.	Contract.	Dilate	Contract. +dilat.
2.00	0	1	0		112.0	
0.10	0	3	0		154.0	
0.06	2	1	67	78.0	227.0	127.7
0.05	1	2	33	74.0	208.5	163.7
0.04	2	1	67	67.5	166.0	100.3
0.03	3	0	100	87.3	—	87.3
0.025	3	0	100	77.3	—	77.3
0.02	3	0	100	75.3	—	75.3
0.013	3	0	100	71.3	—	71.3
0.01	10	2	83	70.6	166.3	86.6
0.004	9	1	90	70.1	172.0	80.3
0.002	6	4	60	70.6	151.1	105.8
0.001	9	1	90	75.8	148.0	83.1
0.0005	6	4	60	66.1	119.8	87.6
0.0002	6	4	60	79.0	133.8	100.9
0.0001	8	2	80	84.1	109.0	89.1
0.00004	7	3	70	76.9	146.5	92.9
0.00002	13	7	65	76.8	135.4	94.1
0.00001	4	6	40	87.0	175.0	139.8
0.000005	2	8	20	73.0	137.6	110.9
0.000003	3	7	30	68.7	152.6	127.4
0.000002						
0	0	21	0	—	153.6	153.6

taneous injection of progesterone and estradiol. It was stated that this reaction was used for the standardization of progesterone crystals and a forthcoming report as to the specificity of the reaction was announced.

It appears from this that two progesterone extracts prepared from the ovaries of swine, and two crystalline preparations of progesterone, made from stigmasterin, produced a distinct effect on the uteri of immature rats under the experimental conditions given in detail above. The action could be observed with quantities of from about 0.3 to 0.02 RB.U. of progesterone.

If a test were subsequently to be required it would be necessary for the various progesterone preparations to produce the action in question from

doses in the same zone. By way of further investigation of this question we employed the technic described above for titration of two synthetic crystalline progesterone preparations, 'Prolution Schering,' and a synthetically manufactured crystalline preparation from Løvens kemiske Fabrik, 'Progesteron' (M.P. 120° C.). The result is shown in tables 7 and 8.

TABLE 8

Dose I.U.	Uterus, macroscopic			Weight of uterus in mg.		
	Total contract.	Total dilat.	% Contract.	Contract.	Dilat.	Contract +dilat.
0.00025	7	3	70	76.7	174.4	106.0
0.000025	10	0	100	79.3	—	79.3
0.0000025	7	3	70	68.1	146.3	123.2
0.00000025	0	10	0	—	142.1	142.1

21-day-old rats given 2γ estradiol benzoate+progesterone (crystalline, Leo) in varying doses. ction down to 0.0000025 Rb.U.

The zones of the action are seen to be quite differently placed than those for the preparations employed previously, both of them being of greater extent and lying between much lower doses. The variation in the position of the zones observed might possibly be thought to cause certain difficulties in the elaboration of the technic described for a quantitative assay of progesterone (especially as regards blood and urine analyses, if, indeed, progesterone be generally found here).

While the lowest dose of the organic preparations and those prepared from stigmasterin to produce certain biological action was from about 0.02 to 0.06 Rb.U., we obtained certain biological action in our rats with 0.000,0025 Rb.U. of the crystalline preparations made from pregnandiol, that is with 1/400,000 of the unit hitherto employed. This quantity is, so far, the smallest weight of highly purified sex hormone to produce biological action.

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THE RELATION OF THE MELANOPHORE HORMONE OF THE PITUITARY GLAND TO OXYGEN CONSUMPTION OF THE RAT[1,2,3]

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THE ACTION of the melanophore hormone of the pituitary gland in dispersing melanin granules of melanophore cells in cold-blooded animals is well known and constitutes an important function of the pituitary gland in such animals as the frog (1). However, in higher animals which do not possess chromatophores comparable with those of cold-blooded animals, the function of this hormone remains obscure. Its presence has been demonstrated in the hypophyses of a wide variety of animals, including man (2, 3, 4). Furthermore, its presence in human urine has apparently been demonstrated (5). Thus, one of the important problems connected with the pituitary is the function of the melanophore hormone in warm-blooded animals.

None of the well-known mammalian effects of pituitary extracts has been shown to be due to the melanophore hormone. Zondek and Krohn (6) reported that their purified erythrophore hormone, intermedin (which is biologically similar to the melanophore hormone but apparently different in certain chemical properties), has no action upon growth or upon the gonads. It does not affect blood pressure, or motility of the uterus, bladder, or intestine, and has no antidiuretic effect. Furthermore, it does not influence the blood sugar, nor does it change the glycogen or fat content of the liver. The substance was also reported to have no influence upon basal metabolism. Van Dyke (7) found no change in the fatty acid content of the liver after injection of a beef melanophore hormone extract. The previous reports of antidiuresis in diabetes insipidus with intermedin (8) were shown to be due to contamination with the antidiuretic principle of the neural lobe (9, 10). Schooley, Riddle and Bates (11) stated that in the pigeon intermedin has no power to sustain weight of the crop sac, thyroid, adrenal, testis, pancreas or liver.

The hormone rapidly disappears from the blood stream of animals after intravenous injection and it cannot be found in other tissues (3, 6). Jores (12) in 1933 reported that instillation of melanophore hormone extracts in the human eye facilitated light to dark adaptation of the eye. However, this was refuted by Buschke (13) and has not been confirmed. Jores and Caesar (14) reported that the melanophore hormone dispersed pigment in the retina of the frog's eye and dilated the pupil. On the other hand, Okamoto (15) claimed that hypophysectomy in the toad did not

[1] Aided by a grant from the Otho S. A. Sprague Memorial Institute and Biological Research funds of the University of Chicago.

[2] Part I of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Chicago.

[3] A preliminary report on some of the material given here has been published (TEAGUE, R. S.: *Proc. Soc. Exper. Biol. & Med.* 40: 516. 1939).

affect pigment change in the retina in light and dark adaptation. Jores (16) reported that intermedin and alkaline posterior lobe extracts produced a fall in temperature and a rise in blood sugar in the rabbit. That this hyperglycemic action was actually due to the melanophore hormone is doubtful in view of the negative report of Zondek and Krohn (6). Furthermore, Geiling and Eddy (17) have shown that the hyperglycemia produced by pituitrin fails to appear if the extract is treated with alkali, which destroys the neural lobe principles but potentiates the melanophore hormone. Thus, no unequivocal pharmacodynamic effect of the substance in higher animals has been demonstrated.

However, recently workers in Collip's laboratory have demonstrated that certain pituitary extracts rich in melanophore hormone produced immediate metabolic stimulation in mammals. O'Donovan and Collip (18) showed that a rise in oxygen consumption and carbon dioxide output with a lowering of the R.Q. resulted from injection of their pituitary extracts in the thyroidectomized as well as in normal rabbits. Such effects of various pituitary extracts have occasionally been seen in the past. O'Donovan and Collip, in reviewing the literature suggested that these effects were due to a 'specific metabolic principle' of the pituitary. The observed metabolic changes were attributed to increased fat metabolism and decreased carbohydrate oxidation. The active principle could be distinguished from the thyrotropic, adrenotropic and growth hormones of the anterior lobe, and from the pressor and oxytocic principles of the neural lobe. It was similar to the melanophore hormone in its chemical properties as well as in its anatomical distribution in the hypophysis; hence, its identity with the melanophore hormone was suggested.

Billingsley, O'Donovan and Collip (19) confirmed the metabolic effects of the extracts in rats and guinea pigs. Metabolic stimulation could be obtained in hypophysectomized and adrenalectomized as well as in thyroidectomized animals. Further studies showed that metabolic stimulation of 27% and a lowering of the R.Q. could be obtained in man with the extracts (20).

Preliminary studies (21) indicate that the melanophore hormone accelerates the tyrosine-tyrosinase oxidase system in the production of melanin in vitro. Since oxygen was utilized in this reaction, it seemed that Fostvedt's studies might constitute further evidence for the identity of Collip's specific metabolic principle and the melanophore hormone, and at the same time suggest a partial explanation for Collip's findings.

However, there are two reports that prompt further investigation of this subject. Firstly, Zondek and Krohn (6) reported that intermedin did not affect the basal metabolic rate. Secondly, Foster and Smith (22) have shown that the reduction in basal metabolism in the rat after hypophysectomy, was entirely due to removal of the anterior lobe, and not to removal of the pars neuralis and pars intermedia. These workers further found that replacement therapy with posterior lobe extracts (presumably including the pars intermedia) did not raise the basal metabolic rate to normal, as did continued treatment with anterior lobe extracts. Thus it seemed desirable to investigate further the effect of the melanophore hormone on oxygen consumption.

METHODS

In these experiments a study was made of certain pituitary preparations in respect to their melanophore hormone content and to their effect on O_2 consumption in normal, hypophysectomized and thyroidectomized rats. Similar studies were also made of the preparations after destruction or inactivation of the melanophore hormone in various ways. The method for assaying

the melanophore hormone by using the hypophysectomized frog as a test object and alkali-treated U. S. P. XI Posterior Pituitary Powder as the reference standard is described in another communication (23). One unit of the hormone corresponds to the activity of 1γ of the reference powder.

Preparation of the extracts. Most of the work reported here was done with 3 extracts. One was a purified melanophore hormone extract prepared by Fostvedt (24) from whale anterior lobe[4] by a modification of Stehle's method (25). Another was a similar extract of beef posterior pituitary[5] made by the same technic. A partially purified melanophore hormone powder made from beef posterior pituitary, known as 'pitmelanin,'[6] was also used. These preparations were usually made up in 0.25% acetic acid in a concentration of 10 mg./cc., brought to boil, and filtered. The extracts were sometimes treated with alkali. In this procedure ½ volume of 2N NaOH was added, and the solution was allowed to stand 24 hours at room temperature, and neutralized with 2N HCl with phenol red for the indicator.

Studies were made with some extracts in which the melanophore hormone was inactivated by various means. In all work of this type, control experiments with hormone-inactivated preparations are highly desirable. For destruction of the hormone by acid treatment, an equal volume of concentrated HCl was added to an extract; and it was allowed to stand at room temperature for 24 hours; then it was neutralized with strong NaOH, and adjusted just acid to litmus with 2N HCl. The procedure for tryptic digestion of the melanophore hormone was as follows: to 40 mg. of a pituitary powder there was added 5 cc. of 1% trypsin, 0.5 cc. of 10% Na_2CO_3, and 4.5 cc. of distilled water. The mixture was incubated at 37° C. for 24 hours, 10% acetic acid was added until the solution was just acid to litmus, and the product evaporated over a water bath down to a volume of 4 cc. Ultraviolet light irradiation was used to inactivate the hormone in some experiments. The extracts were placed in a beaker and given 4 to 5 exposures of 60 minutes each to a mercury vapor arc lamp at 30 cm. distance over a period of 2 to 3 days. Inactivation by treating extracts with H_2O_2 was accomplished by simply adding 30% H_2O_2 to the extract and allowing it to stand 24 hours at room temperature. Enough H_2O_2 was added to make a total concentration of 3 to 10%. In every case, the extract was checked for absence of melanophore hormone by injection in a hypophysectomized frog. Extracts of beef liver, muscle and kidney were prepared for control studies by taking up the acetone-dried powder with 0.25% acetic acid at a concentration of 10 mg./cc., boiling 1 minute, and filtering.

Methods of metabolic studies on rats. Both albino and hooded rats were used. The diet consisted of bread, corn meal, powdered milk mixture and raw beef lung, supplemented with lettuce. Hypophysectomy in rats was performed by the parapharyngeal approach, as originally described by Smith (26). Thyroidectomy was carried out with the aid of a dissecting microscope.

[4] In the whale all the melanophore activity is in the anterior lobe (GEILING, E. M. K.; *Bull. Johns Hopkins Hosp.* 57: 1. 1936).

[5] Generously supplied by the Wilson Laboratories, Chicago.

[6] Obtained through courtesy of Parke, Davis & Co., Detroit.

The parathyroids were often removed also, and tetany was prevented by feeding calcium lactate (1%) in drinking water. In both operations, success was judged by a depression of B.M.R. and the operations were checked at necropsy by gross and histologic observations. Autopsies showed that incomplete operation occurred in only one case, that of one of the thyroidectomized rats. At least 3 weeks elasped before the operated animals were used in the experiments.

Rats both recently fed and fasted 12 to 18 hours were used for O_2 consumption studies. The O_2 consumption was measured in a modified Benedict spirometer with $CaCl_2$ used to absorb the expired water vapor, and soda lime to absorb the carbon dioxide. The container for the rat was an ordinary chemical desiccator which was immersed in a water bath thermostatically maintained at a temperature of 28° C. Metabolic rates were calculated in terms of liters of O_2 consumed per square meter body surface per 24 hours. The surface area was calculated from the fasted weight using Rubner's formula (27) of $9.1 \times Wt.^{2/3}$.

The usual procedure was to place the rat in the desiccator for 2 to 3 hours for a basal control reading. Thereafter, the rat was removed, injected intraperitoneally, and replaced in the desiccator. A continuous O_2 consumption record was taken for the following 5 to 9 hours. Often the metabolism was again measured the next morning. After a few preliminary trials the rats kept quite still during the greater part of the experiment. In calculating the O_2 consumption, care was taken to utilize only perfectly regular tracings of at least 30 minutes' duration.

RESULTS

The average depression of basal O_2 consumption in the thyroidectomized rats was -23.2%, and in the hypophysectomized rats -34.6%. Comparable results with the extracts were obtained in fed or fasted, normal, hypophysectomized and thyroidectomized rats; that is, an extract possessing metabolic stimulating action would show a response in all types of animals.

In figure 1 are shown the results of control experiments. The highest metabolic rates following injection in each experiment were averaged. The majority of the control results fell within an increase of 10%; there was, however, considerable variation. In the normal control experiments and in the saline experiments there were 2 cases out of 24 in which the metabolic rise during the day reached +20%. Occasionally with the extracts of liver, muscle, and kidney, an increase up to 30% was seen.

In figure 2(N) is shown a representative graph of the normal metabolic variations during an afternoon, using the figures obtained in the morning as a basis. A typical result with saline injection is essentially similar.

Figure 2 (C) shows the type of response obtained with a large dose (7 to 8 cc./kg.) of Collip's Extract 622[7] made from beef anterior pituitary and found here to contain 10,000 U/cc. of melanophore hormone. The best results, both as to intensity and regularity of metabolic response, were obtained with

[7] Obtained through the courtesy of Dr. J. B. Collip.

this extract, which undoubtedly contains a substance that stimulates metabolism. The effect was not obtained with smaller doses as is shown in figure 3 (B and C). These results are similar to those reported by O'Donovan and Collip (18). However, it is significant that a purified melanophore hormone preparation made from whale anterior lobe and assaying 10,000 u/mg., usually failed to produce metabolic stimulation when given in 10 to 20 mg./kg. doses, or 25 times the dose of Collip's Extract 622 (fig. 3, G). In 3 cases there was a rise of over 20%, in 1 case a fall below 30% occurred, while

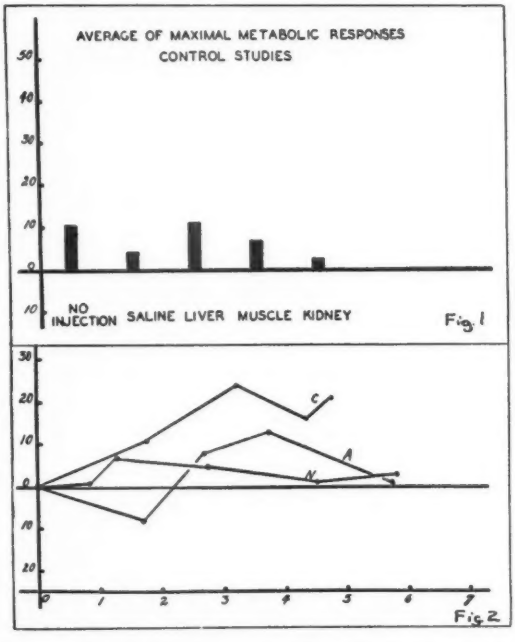

Fig. 1. PERCENTAGE CHANGE IN O_2 CONSUMPTION OVER BASAL VALUE FOLLOWING INJECTION. In experiments with beef liver, muscle and kidney, 10 mg. of each injected. Each average represents at least 7 experiments. Fig. 2. REPRESENTATIVE METABOLIC CHANGES FOLLOWING INJECTION. Abscissa: time in hours following injection at 0. Ordinate: percentage change in O_2 consumption, N, normal control. C, effect of 8 cc./kg. of Collip's Extract 622. A, effect of 8 cc./kg. of Armour & Co. anterior pituitary preparation.

7 other experiments were within a range of normal variation. In these experiments the metabolic response did not parallel the melanophore hormone content of the extracts. This, together with other findings, throws doubt upon the identity of the melanophore hormone and the specific metabolic principle of Collip and coworkers.

An extract[8] prepared similarly to Collip's Extract 622 containing 2000 u/cc. of melanophore hormone failed to increase the metabolism in doses of 3 to 8 cc./kg., in 5 cases out of 6 (fig. 3, D, E).

[8] Kindly supplied by Dr. R. L. Kutz.

In another series of experiments, partially purified melanophore hormone made from beef posterior pituitary, assaying 1000 U/mg., was used (fig. 3, F). Doses of 60 to 90 mg./kg. gave a variable response. In 2 cases the metabolic rise was over 20%, but in 3 others there was no response, so that the average rise was less than 20%.

Crude posterior pituitary powder containing 1000 U/mg. of hormone, in a dose of 4 to 8 mg./kg., gave no significant change in metabolism. After alkali treatment in which the posterior lobe principles were destroyed and

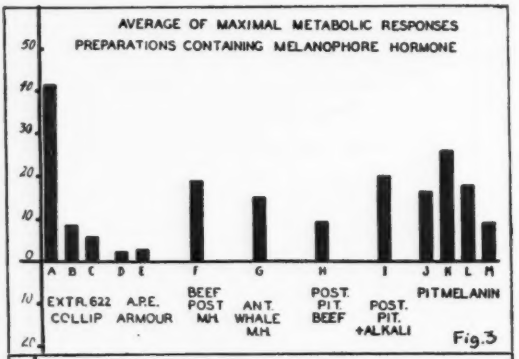

Fig. 3. Percentage change in O_2 consumption over basal value following injection. A, 7 to 8 cc./kg. B, 3 to 4 cc./kg. C, 0.7 to 0.8 cc./kg. D, 7 to 8 cc./kg. E, 3 to 4 cc./kg. F, 60 to 90 mg./kg. G, 10 to 20 mg./kg. H, 4 to 8 mg./kg. I, 4 to 8 mg./kg. J, 200 mg./kg. K, 70 mg./kg. (alkali-treated). L, 70 mg./kg. M, 35 mg./kg. Fig. 4. Percentage change in O_2 consumption over basal value following injection of inactivated pitmelanin in 70 to 150 mg./kg. doses.

the melanophore hormone potentiated, injection of the substance in a similar dosage gave one significant change out of 3 experiments (fig. 3, H, I).

Pitmelanin (Parke, Davis & Co.) which is a melanophore hormone powder prepared from beef posterior pituitary, assaying 1000 U/mg., gave insignificant changes in 35 mg./kg. doses (fig. 3, M). With 70 mg./kg. there were 2 increases over 20% and 2 below (fig. 3, L). With the same dosage of alkali-treated material, 3 trials were over 20% and 4 showed no significant change (fig. 3, K). However, on increasing the dose to 200 mg./kg. the response

was lessened (fig. 3, J). The averages likewise show that the only significant response is at 70 mg./kg. level with the alkali-treated material (fig. 3, K). Here, again, the metabolic response failed to follow the melanophore hormone content; hence it cannot be said that the latter is an index to the metabolic stimulating effect of an extract.

The results of injections with inactivated melanophore hormone preparations also did not lend support to the idea that the melanophore hormone and the metabolic stimulating substance are identical.

The averages of these results are shown in figure 4. Destruction of the melanophore hormone was assured in every case by injection into a hypophysectomized frog. Treatment of pitmelanin with concentrated HCl definitely destroyed the melanophore hormone, and yet in 2 experiments an

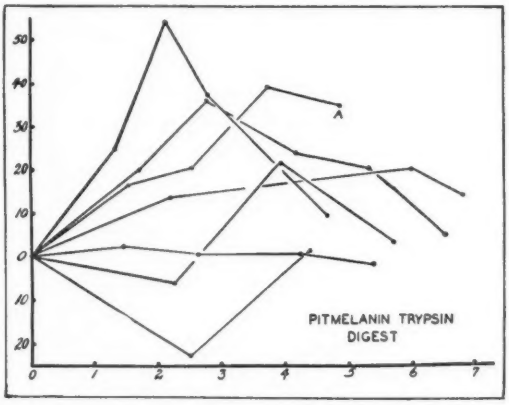

Fig. 5. METABOLIC RESPONSES OBTAINED AFTER TRYPSIN DIGESTION OF PITMELANIN IN 70 TO 150 mg./kg. DOSES. Numerals as in Fig. 2. A, trypsin digest of beef posterior pituitary, 20 mg./kg.

increase in O_2 consumption was still obtained. The resulting product (neutralized) after acid treatment proved toxic to the animals. One of the rats died 1 hour after injection. Trypsin-digested pitmelanin containing no melanophore hormone, produced definite metabolic stimulation in doses of 70 to 150 mg./kg. (fig. 4, 5). It is to be noted that a more consistent metabolic response was obtained with the trypsin-inactivated pitmelanin than with the original extract. In this case, also, metabolic responses were unrelated to the melanophore hormone content. The digested product was not toxic in the dosage used.

After ultraviolet light irradiation of pitmelanin, both the melanophore hormone and the substance responsible for metabolic stimulation are inactivated (fig. 4). The possibility of oxidation by the ultraviolet light treatment was not eliminated, since the extracts were irradiated in open beakers. The resulting product gave an increase of less than 20% on 2 occasions and decreased the metabolism in 8 other trials.

Pitmelanin treated with H_2O_2 likewise lost both melanophore-expanding property and metabolic stimulating effect, as seen from figure 4.

DISCUSSION

In the experiments described above, the only pituitary preparation that consistently stimulated metabolism was Collip's extract. The amount of melanophore hormone contained in the dose of the extract necessary to stimulate metabolism, was entirely out of the (theoretical) physiologic range, 2000 times the amount necessary to darken a frog, when the dose per body weight is compared. Definite indication that the metabolic stimulation produced by Collip's extract is not due to its melanophore hormone content is shown by the absence of a metabolic response with extracts containing equivalent amounts of the hormone, obtained from whale anterior lobe or beef posterior lobe. Furthermore, pitmelanin produced greater metabolic stimulation than did whale anterior lobe preparations, although it contains only 1/10 the melanophore hormone activity of the latter. The fact that the very highly purified whale anterior lobe products, rich in melanophore hormone exhibited the least metabolic effect seems to be good evidence that the metabolic stimulation obtained from pituitary extracts is due to some product other than the melanophore hormone.

Further evidence for this conclusion is found in the experiments with inactivated extracts. More consistent metabolic stimulation was seen with pitmelanin after HCl treatment or tryptic digestion than with the original extract. The toxic action seen in the case of the HCl-treated extract suggests the possibility that the metabolic stimulation produced by certain pituitary extracts might even be non-specific, in that a substance such as a protein breakdown product might be the responsible agent. The finding that ultraviolet light and H_2O_2 treatment destroyed both the melanophore hormone and the substance responsible for metabolic stimulation need not be inconsistent with this suggestion.

During the course of the preparation of this paper there appeared a note by Denstedt and Collip (28), who stated that pituitary fractions had been obtained in which the melanophore hormone and the metabolic principle apparently do not parallel one another. Hence, it can be accepted that the melanophore hormone is not identical with the specific metabolic principle reported by Collip and coworkers. Further search will have to be made to ascertain the rôle of the melanophore hormone in warm-blooded animals. Various efforts are now being made in an attempt to solve this problem.

SUMMARY

Preparations of the pituitary gland rich in melanophore hormone, obtained from various sources and prepared by different methods, vary considerably in their effect on O_2 consumption in rats. Hypophysectomized or thyroidectomized rats are subject to the same variations in response as normal animals.

A pituitary extract of Collip and a melanophore hormone extract pre-

pared from whale anterior lobe, although having a similar melanophore hormone content, showed marked differences in their action on O_2 consumption in rats, in that Collip's extract in large doses was capable of stimulating metabolism while the whale preparation was inactive.

When the melanophore hormone in pitmelanin was destroyed by HCl or by tryptic digestion, the product produced even more consistent metabolic stimulation than the original extract.

The evidence here warrants the conclusion that the melanophore hormone of the pituitary gland is not identical with a substance in pituitary extracts which would increase the B.M.R. This statement finds support in the very recent report of Denstedt and Collip. The data are insufficient to demonstrate the presence of a specific metabolic principle of the hypophysis, since metabolic stimulation was produced with a pituitary extract after treatment with acid and after tryptic digestion, and since metabolic responses were occasionally seen with extracts of muscle, liver and kidney.

The author wishes to express his appreciation for valuable advice and criticism to Dr. E. M. K. Geiling of the Department of Pharmacology, under whose direction this work was completed.

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BIOLOGICAL ASSAY OF THE MELANOPHORE HORMONE OF THE PITUITARY GLAND[1,2]

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DURING A STUDY of the effect of melanophore hormone preparations upon the oxygen consumption of rats (1), it was found that an accurate assay of the hormone was necessary. Since no established, reliable method for determining its potency has been described, and in view of the recent interest in this hormone, it seemed essential to devise a dependable method and to establish a unit of activity. The hypophysectomized frog was chosen as a test object for the assay, since this preparation has been shown to give a specific response to the melanophore hormone (2). It was found that this subject is somewhat more sensitive than the normal, light-adapted frog, in that skin darkening could be obtained in the hypophysectomized frog with doses that were too small to affect the latter. Furthermore, the response is more reliable in the operated animal, because less control of environmental conditions needs to be exercised. It also seems more physiologic to test the hormone on an intact animal rather than on the isolated frog skin as suggested by Trendelenburg (3). The best results were obtained with frogs (*Rana pipiens*) weighing 30 to 40 gm. Hypophysectomy in the frog is a simple procedure and has been described previously (2, 4).

In all biological assay work it is desirable to compare the activity of the unknown with reference to a standard (5). For this purpose, the United States Pharmacopoeia (U. S. P.) XI Posterior Pituitary Powder was chosen because the method of collecting the glands and preparing the powder makes it probable that the hormone content of this preparation is most constant. The action of the melanophore hormone on frog melanophores is known to be potentiated by at least 10 times when treated with alkali (6). Hence, to avoid confusion, and to detect smaller amounts of the hormone, the reference standard and the unknowns were always treated with alkali.

The method of preparing the standard stock solution is as follows: the posterior pituitary powder is extracted with 0.25% acetic acid and made up to 1 mg./cc. according to the directions in the U. S. P. XI. One-half volume of 2N NaOH is added and the resulting solution allowed to stand at room temperature for 24 hours. Two-normal HCl is added until the solution is just alkaline to phenol red and 0.1N HCl is used to make the solution acid

[1] Aided by a grant from the Otho S. A. Sprague Memorial Institute, and Biological Research Funds of the University of Chicago.

[2] Part II of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Chicago.

to phenol red. Physiologic saline is then added so that the final concentration is ⅓ mg./cc., and the final pH of the solution is about 6.8. The solution must be kept in the dark at 4° C. With some pituitary preparations a gelatinous precipitate settles out with this treatment. This precipitate is devoid of melanophore activity.

Through extensive trial it was determined that, in general, the injection of 0.001 mg. (1 γ) of the alkali-treated powder produced in about 2 hours in a 30 gm. hypophysectomized frog, a definite submaximal darkening with stellate melanophores, fading in around 6 hours. This amount of the reference standard may conveniently be designated as one unit of melanophore hormone. Some biological variation is encountered, hence at least 6 frogs should be used in an assay. The sensitivity of the method is such that 1/10 of a unit, 0.0001 mg. (0.1 γ) per 30 gm. frog produces no gross color change and rarely any microscopic evidence of melanophore expansion; while 10 U, 0.01 mg. (10 γ) per 30 gm. frog produces a maximal color change, an intense blackening of the skin with completely reticulate melanophores, fading in about 20 hours. Thus for the sake of convenience and in conformity with the rule of Burn (5) that submaximal doses be used in biological assay, a unit of melanophore hormone is defined as the activity of 1γ (0.001 mg.) of alkali-treated U. S. P. XI Standard Posterior Pituitary Powder, when injected into a hypophysectomized frog in a dosage of 1γ per 30 gm. frog.

Two important considerations must be observed in the procedure. The frogs tend to become more sensitive to the hormone after 1 to 2 weeks following hypophysectomy. Consequently, better results will be obtained by waiting at least a week after the operation before using the frogs, Furthermore, the frogs must be kept in water at 15° to 20° C. in order to obtain consistent results. At higher temperatures the melanophore reaction is more rapid but the frogs are not so sensitive.

In an assay, the alkali-treated preparations are injected through the floor of the mouth into the ventral lymph sac. In the case of the stock solution, the concentration of ⅓ mg./cc. makes it convenient to take of a 1:100 dilution, a dose in tenths of a cc., equal to the weight of the frog in gm., in order to give the unit dose of 1γ per 30 gm. frog. The dilution should be made on the day of the assay. Under the conditions described, the maximal response occurs about 2 hours after injection. Observations of the skin should be made grossly and checked by examination of the web melanophores under a suitable magnification, such as a dissecting microscope. Proper dilutions are made to equate the unknown and the standard at the 1-unit level, and the results are expressed in units, the reference standard containing 1000 U /mg.

The U. S. P. Posterior Pituitary as a dry powder was found to retain its potency over a period of 8 months. A dry purified melanophore hormone preparation containing 10,000 U/mg. was found to deteriorate to 100 U/mg. in the course of 3 months. The standard stock solution in ⅓ mg./cc. concentration is reliable for only 2 weeks, after which it slowly deteriorates. More dilute solutions are not reliable.

SUMMARY

A method for the biological assay of the melanophore hormone of the pituitary is described, using the hypophysectomized frog as the test object and alkali-treated U. S. P. Posterior Pituitary Powder as the reference standard. A unit of melanophore hormone is defined as the activity of 1γ of the alkali-treated standard, when injected into a 30 gm. hypophysectomized frog.

The author wishes to express his appreciation for valuable advice and criticism to Dr. E. M. K. Geiling of the Department of Pharmacology, under whose direction this work was completed.

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A STUDY OF THE FUNCTIONAL INNERVATION OF THE HYPOPHYSIS

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THERE IS MUCH clinical and experimental evidence which indicates that there exists a functional nervous connection between the brain and the anterior lobe of the hypophysis. From the clinical side one may recall the numerous instances of thyroid, ovarian and uterine dysfunction which seem to be of neurogenic origin, disturbances which may ultimately be traced to hypophyseal disorders. Experimentally the work of Schweizer et al. (1) showing the loss of the cyclic behavior of the ovary after homoplastic anterior pituitary grafts in the guinea pig, as well as numerous experiments on stalk section by other workers indicate the importance of the normal anatomical relations of the hypophysis.

Several important contributions to our knowledge of the detailed anatomy of the nerve connections between the hypophysis and central nervous system have been made in the past few years. However, in spite of these advances the functional importance of these several nervous pathways still remains obscure.

In the rabbit one finds an animal which is admirably suited to the rôle of biological indicator in investigating this question. It is well known that in this species ovulation is not spontaneous as in most mammals, but is dependent for its initiation on the act of coitus. It is believed that an intact hypophyseal innervation is necessary to bring about the ovulatory response to copulation.

The varied nervous pathways which have been suggested as factors in this connection are: (*a*) a nervous pathway to the hypophysis by way of the cervical sympathetics (2); (*b*) by way of nerve fibers of the stalk which in some species have been shown to enter the anterior lobe or pars tuberalis directly (3–6); or by means of stalk fibers which pass to the pars nervosa of the hypophysis which then activates humorally the anterior lobe (7) and (*c*) by way of parasympathetic fibers taking origin from the facial nerve and joining the carotid group of hypophyseal fibers (7).

In regard to the first of these suggestions it has been conclusively shown that destruction of the cervical part of the sympathetic trunk does not interfere with ovulation and conception in the rabbit (7–10); thus removing the sympathetic from consideration as the pathway for this impulse.

The second possibility, that of a direct hypothalamic pituitary connection as the functional pathway, certainly has evidence in its favor. Harris (11), Hinsey (12) and Brooks (10) have carried out stalk sectioning experiments and have been unable to obtain ovulation in the operated animals. These results suggest that herein lies the answer to this vexing problem. The one obvious feature which detracts from the conclusiveness of these experiments is the fact that the procedure inevitably produces considerable vascular disturbance in the hypophysis, and such a change cannot be excluded from consideration as a possible explanation of the results. However,

Brooks obtained ovulation in animals with partially sectioned stalks; these animals showed as much circulatory disruption as did those animals with completely sectioned stalks which did not ovulate.

If such a functional connection does exist it still is not clear whether it is a direct one to the anterior lobe of the hypophysis or indirect by way of the posterior lobe followed by an endocrine influence on the anterior lobe as suggested by Hinsey and Markee. The authors do not subscribe to the latter possibility, but it is to be remarked that the possibility of such a mechanism cannot be excluded.

In regard to the third possibility, evidence is at hand to suggest that some of the nerve fibers which pass from the carotid plexus to the hypophysis are of other than cervical sympathetic origin (5, 10). Chorobski and Penfield (13) have demonstrated the existence of a bundle of small myelinated and non-myelinated fibers which forms a part of the nervus intermedius of Wrisberg, but which leaves the facial nerve as part of the greater superficial petrosal and joins the carotid plexus. Hinsey and Markee (7) have suggested this as the possible pathway to the hypophysis for impulses initiated during the act of copulation. This possibility has indirect anatomical evidence in its favor when one recalls that the visceral efferent portion of the facial nerve supplies a region in the pharynx which lies in approximation to the site of differentiation of Rathke's pouch. It was to investigate the existence of such connections that the work reported in this paper was carried out.

Stimulation of the Seventh Nerve

Method. In this series of experiments adult non-pregnant rabbits were used. Ether or urethane anesthesia was employed. An incision was made below and behind the ear, the cartilaginous portion of the meatus was loosened from its bony attachments and the entire auricle reflected forward. The facial nerve was then identified and the nerve sectioned about a centimeter distal to its exit from the stylo-mastoid foramen. Using rongeurs and cutting probes the entire lateral wall of the middle meatus was dissected away. With the exposed nerve as a guide the thin lateral wall of the facial canal was removed. Thus the nerve was exposed in its course along the medial wall of the meatus to within a millimeter or two of the genu of the nerve. A unipolar electrode of fine wire was then placed in the canal, pushed in the direction of the genu and the nerve stimulated. Following stimulation, the middle meatus was packed with absorbent cotton and bone wax and the wound closed. The animals were allowed to recover from the anesthetic and were then killed from 12 to 36 hours after the operation. The ovaries were examined both macroscopically and microscopically.

The electrical stimulus was obtained from an inductorium, the minimum strength of current being determined as the amount that would produce contraction of the facial muscles on stimulation of the distal end of the severed nerve when the second electrode was attached to the tongue or upper lip. In some experiments the intensity of the current was increased, in one instance to such an extent that current spread produced contraction of the levator scapula and trapezius muscles.

The duration of stimulation was not constant in all animals, the variation ranging from 3 minutes continuous stimulation in one case, to 30 seconds stimulation alternating with a 2-minute rest over a 30-minute interval in another.

Results. Seven animals survived the operative procedure and remained in fair condition until they were killed. All but 2 of these animals showed nystagmus and marked disturbance of equilibrium due to damage to the vestibular apparatus during the bone resection. Examination of the ovaries of all 7 animals gave neither macroscopic nor microscopic evidence of ovulation. The follicles were well formed but showed no development beyond that of the control animals.

The only evidence available that the stimulus was producing a response in the areas supplied by the visceral efferent part of the nerve was the apparent accumulation of rather large amounts of mucus in the throat after the onset of stimulation. Several animals developed mucus plugs and died because of this. It cannot be definitely stated that this secretion was a result of stimulation, but this is suggested since it occurred in animals receiving non-volatile as well as those receiving volatile anesthetics.

Bilateral Avulsion of the Facial Nerve

Method. Surgical attempts to bring about destruction of the facial nerve proximal to the geniculate ganglion are fraught with many difficulties. This is particularly true in experiments which necessitate maintenance of the animal in good condition for several weeks post-operatively. Intracranial approach to the roots of the facial nerve is possible, but technically difficult. The writers attempted destruction of the nerve by an approach through the tympanic cavity. The principal drawback to this method lies in the fact that the incidental disturbance to the vestibular apparatus leads to marked postural disturbance, which, coupled with the effects of the facial paralysis, makes the animals useless for subsequent breeding purposes.

It was learned by the authors that if sufficient care was taken a suitable nerve destruction could be accomplished without entering either the cranial or tympanic cavities. To accomplish this the seventh nerve was exposed through a small incision immediately below the ear. The epineurium was loosened from its periosteal attachments at the styloid opening. The nerve was then grasped with a small hemostat, sectioned distal to the clamp and the proximal part avulsed by slow traction. In most instances immediately after avulsion there was a noticeable flow of spinal fluid through the opened styloid foramen. In some animals about 0.1 cc. of 95% alcohol was then injected into the canal by use of a No. 27 platinum-iridium needle, in others this added precaution to secure complete nerve destruction was not taken. The facial canal was then plugged with sterile bone wax and the wound repaired.

The removed segments of nerve were fixed in formalin, embedded in paraffin and sectioned serially at 10μ. If, on examination of the sections, the geniculate ganglion could be identified, and if the nerve was intact for 4 or 5 mm. proximal to the ganglion, the avulsion was judged complete.

Post-operatively many of the animals did not do well. The paralysis of the buccinator muscle made feeding difficult, and a plug of food had to be removed from the oral vestibule several times a day in an attempt to keep them in a fair state of nutrition.

Attempts were made to breed the animals 5 to 10 days after the operation and several times thereafter. Some animals were killed in 30 to 40 days while others were allowed to survive for 60 days or more. When killed they were examined for pregnancy, the ovaries, adrenals, thyroid and hypophysis removed and preserved for histological study.

A second small series was operated for controls. In these animals the facial nerve was exposed bilaterally, and about a centimeter of the nerve trunk was excised just after its exit from the foramen.

Results. In the experimental series 18 animals were obtained in which avulsion was considered complete as judged by the criteria given above. Of these 18 animals, 7 became pregnant. Four of these gave birth to normal litters and the other pregnancies were discovered at autopsy. The earliest pregnancy noted was in an animal which delivered on the 36th post-operative day; the latest in an animal which delivered on the 83rd day, after 7 unsuccessful attempts at breeding had been made. Of the remaining 11 members of this group all showed marked loss of weight (from 460 to 1200 gm.) in spite of efforts to maintain their nutrition.

In the control series of 5 animals, 2 gave birth to normal litters; the others failed to become pregnant and showed the same weight loss as the non-pregnant animals of the first group.

Microscopic examination of the hypophysis, thyroid and adrenal glands of these animals failed to show any significant histological difference between experimental and control groups. These examinations were not absolutely complete since cell counts in the hypophysis were not made.

DISCUSSION

Of the 3 possible pathways by which the hypophysis may be placed under control of the central nervous system only one has experimental evidence in its favor. That is a direct connection between the hypothalamus and pituitary by way of fibers of the infundibular stalk. Of the 2 remaining possibilities, the sympathetic route has been excluded previously, leaving only parasympathetic connections through the facial nerve as uninvestigated.

The authors have attempted to test the importance of the parasympathetic part of nerve VII in the following ways: (*a*) direct stimulation of the nerve in the region of the geniculate ganglion in an attempt to induce ovulation; (*b*) bilateral destruction of nerve VII combined with tests for the normal ovulatory response to copulation.

In the first instance 7 animals were successfully carried through the experimental procedures. In not one of these animals were either micro- or macroscopic ovarian changes produced which would indicate increased pituitary activity following stimulation. Such negative results are not conclusive proof of the absence of nervous connections by way of the facial nerve for the obvious reason that in using electrical stimuli one is only guessing at the frequency, intensity and duration of stimulus which might be necessary to produce a response in the gland. However, in view of the work (14, 15) in which ovulation was induced by direct stimulation of the brain and hypothalamus, it is likely that if the pathway was the route under investigation,

the more localized stimuli applied in these experiments should have been effective.

In the second series of experiments the facial nerve was destroyed bilaterally. In this group ovulation was obtained in 7 of 18 animals or 39% of the series. In a small control group of 5 animals, in which simple nerve section was performed, ovulation was obtained in 2 of 5 animals or in 40% of the series.

The rabbit is a relatively easy animal to breed under laboratory conditions and a high percentage of conceptions could be expected in healthy animals. However, the rabbit is sensitive to slight changes in nutrition and under adverse conditions often does not breed readily. The rather low percentage of pregnancies in the above series can be attributed to nutritional disturbance brought on by the facial paralysis, and in view of the close correlation between results obtained by control and experimental groups it can be concluded that the destruction of the facial nerves had only a secondary effect on the failure to produce pregnancy in the operated animals.

The above results seem to exclude the facial nerve from consideration as a pathway for impulses from the central nervous system to the hypophysis. However, it should be pointed out that in view of the complicated relations between the branches of the facial, glossopharyngeal and vagus nerves as they pass through the temporal bone these latter 2 cranial nerves cannot be definitely excluded from consideration. Combined destruction of nerves VII, IX and X may serve to answer this question.

In view of the experiments of Harris (11) and particularly those of Brooks (10) the authors are inclined to believe that fibers of the infundibular stalk serve as the functional connection between the brain and the anterior lobe of the hypophysis.

SUMMARY

The functional relation between the parasympathetic component of the facial nerve and the hypophysis has been investigated by (*a*) electrical stimulation of the nerve at the geniculate ganglion and (*b*) bilateral destruction of the facial nerve. Stimulation of the facial nerve at the geniculate ganglion did not produce ovulation in adult rabbits. Bilateral destruction of the facial nerve did not prevent the normal ovulatory response to copulation.

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EFFECTS OF HYPOPHYSECTOMY UPON THE BLOOD PICTURE OF THE RAT

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THE INITIAL REPORT of Houssay and his coworkers (1) on anemia following hypophysectomy in the rabbit, together with Cushing's description (2) of an erythremia as part of the basophilic syndrome, stimulated a number of experimental investigations into the effects of pituitary disturbances on the blood picture (3, 4, 5). In earlier work a hematological significance has been attributed to the thyroid (6–10) and the adrenal cortex (11, 12, 13), while more recently the importance of the anterior pituitary has been emphasized.

Meyer, Stewart, Thewlis and Rusch (14) concluded that hypophysectomy in rats resulted in an anemia. Flaks, Himmel and Zlotnick (15) reported a thermostable anterior pituitary fraction which stimulated erythrocyte production in rats. Overbeek (4) failed to find any anemia in rats after hypophysectomy. Overbeek and Querido (16), however, described a decrease in numbers of erythrogenic elements in the bone marrow of hypophysectomized rats. More recently these same authors (30) have found that the reticulopenia following hypophysectomy may be prevented by using a heat labile fraction of the pars anterior. The contradictory results of experimental investigation and the inconclusive nature of the clinical evidence (17, 18) appeared to warrant a re-investigation of the relationship of the pituitary to the blood picture. The present work is concerned with a description of the changes in hemoglobin and the counts of reticulocytes, erythrocytes and leucocytes in hypophysectomized rats. In addition some observations on histological modifications in the bone marrow are included.

MATERIALS AND METHODS

Male and female rats, weighing 150 to 230 gm., were employed. Hypophysectomy was performed by the parapharyngeal approach, following ether anesthesia. Only completely hypophysectomized animals were employed. All animals which had been incompletely hypophysectomized, as judged by careful examination of the sella turcica contents at the time of autopsy, were not included. The animals were kept in a constant temperature room and fed a balanced mixed diet, containing liberal quantities of fresh lettuce and carrots. A 20% sucrose solution was substituted for

[1] Submitted in partial fulfillment of the requirements for the degree of Master of Science, New York University.

drinking water. Two types of controls were used. The animals in one series were subjected to operations in which the surgical procedure was identical with that of hypophysectomy, except that the pituitary was not removed. In another series a group of normal unoperated animals was kept on a reduced diet of 6 gm. of ration per animal per day (19). This resulted in a 20 to 40% loss of body weight, which is comparable to the loss sustained by hypophysectomized animals. Total red and white blood cell counts, differential leucocyte counts, hemoglobin determinations and reticulocyte counts were made at weekly intervals. In addition, sections of bone marrow were prepared for histological examination.

Total erythrocyte counts were made in duplicate, on peripheral blood obtained from the tail. Each of the observers counted the cells on 200 squares of a Levy-Hausser hemacytometer chamber. Counts were required to agree within ±4%. Hayem's solution was used as the diluting fluid.

The total leucocyte counts were obtained in a similar way. Each observer counted the cells on 5 of the square millimeter areas of the hemacytometer ruled surface. All counts were required to agree within ±10%. The diluting fluid was a 2% acetic acid solution.

The reticulocyte counts were made on wet smears from whole blood previously diluted with 2% sodium citrate and stained with brilliant-cresyl-blue. One thousand erythrocytes were counted for each determination, and the reticulocytes expressed as a percentage.

Hemoglobin determinations were made with a micro-Dubosq type colorimeter. The blood of one of the investigators (*E.V.*) was used as the standard. His blood was drawn up to the one-tenth mark of a hemoglobin pipette and diluted with 5 cc. of distilled water. The diluted blood was then saturated with carbon monoxide by bubbling illuminating gas through it for 2 minutes. Similarly treated samples from the experimental animals were then matched against the standard and the hemoglobin expressed as a percentage.

The differential counts were determined on dried blood smears treated with Wright's stain.

The histological examinations of the bone marrows were carried out on material obtained from the right femurs. The bones were cut at both the proximal and distal ends, fixed in Bouin's solution, decalcified in phloroglucin-nitric acid, and sectioned in paraffin. Hematoxylin-eosin, or Kingsley's stain was used.

RESULTS

The behavior of the erythrocyte counts in the animals of the 3 experimental groups, over a period of 6 to 9 weeks, is indicated in figure 1. Thirty-eight normal rats, taken at random from our colony, showed a mean erythrocyte count of 8.35 million cells per cu. mm., with a variation of 7.9 to 9.1 million per cu. mm. As can be seen from this graph, the weekly mean count of the hypophysectomized rats (Curve A) dropped steadily for 6 weeks, reaching a value of 5.7 million per cu. mm. at the end of this time. Thereafter, the mean fluctuated between 5.0 and 6.0 million per cu. mm. up to the 9th week. The weekly mean of the operated control group (Curve B) dropped only slightly below normal for 2 weeks after the operation, but from the 3rd week on, assumed normal values. Curve C indicates that animals placed on a restricted diet showed a slight erythremia which was maintained for the

duration of the experiment.[2] Individual variations caused the erythrocyte counts of animals in the hypophysectomized and operated control groups to overlap during the first 2 weeks after operation. By the 3rd week, however, there was already a difference of 0.5 million per cu. mm. between the highest individual count in the hypophysectomized group and the lowest in the

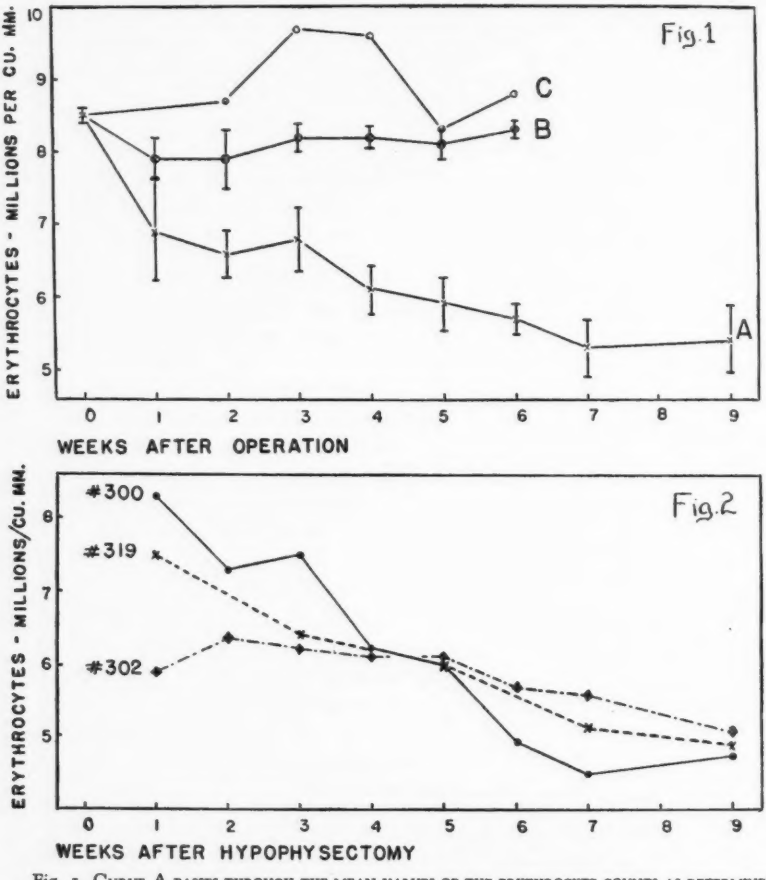

Fig. 1. Curve A passes through the mean values of the erythrocyte counts as determined in a total of 23 completely hypophysectomized animals. Curve B indicates the mean values of the erythrocyte counts of 11 operated controls. Curve C represents the mean values of the erythrocyte counts of 5 rats placed on the starvation diet. The vertical lines drawn through the mean values in curves A and B represent the limits of twice the average deviation from the mean. Fig. 2. Curves showing the weekly erythrocyte counts of 3 typical hypophysectomized rats for 9 weeks after operation.

operated control group. This difference became 1.2 million per cu. mm. in the 4th week and 2.0 million per cu. mm. in the 6th week.

[2] Although the graphs for the starved normal and operated control groups stop at 6 weeks, counts taken from several animals 6 weeks beyond this time indicated the same general trend.

Figure 2 shows the weekly erythrocyte counts for 3 typical hypophysectomized animals. It will be noted that whereas the intensity of the drop in erythrocyte count varied considerably during the 1st week after hypophysectomy, the counts for the 3 animals tended to reach approximately the same level by the 9th week.

Figure 3, which indicates the mean hemoglobin percentages for the 3 groups, closely resembles figure 1. Fourteen normal rats of the colony showed a variation of 98 to 111%, with a mean of 104%. Whereas the mean hemoglobin values for the operated control group fluctuated slightly below normal for 6 weeks and the values for the normal starved group again indicated erythremia, the hemoglobins of the hypophysectomized animals fell steadily

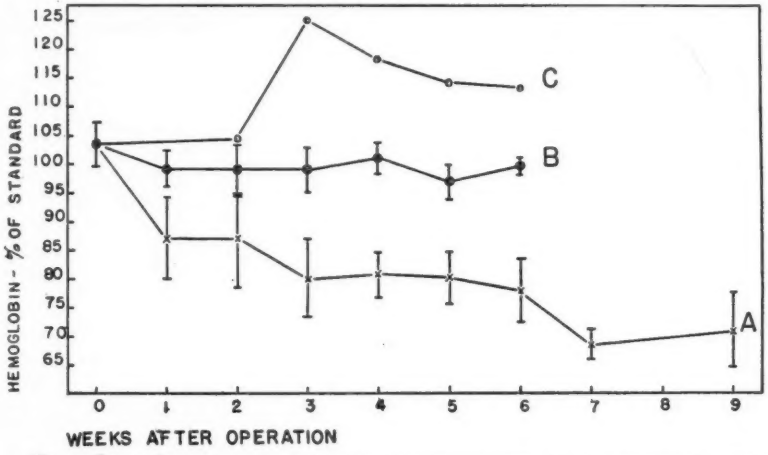

Fig. 3. Curve A passes through the mean hemoglobin values as determined in a total of 23 completely hypophysectomized rats. Curve B passes through the mean hemoglobin values obtained in 11 operated controls. Curve C represents the mean hemoglobin values in 5 rats placed on a starvation diet.

towards a plateau of 65 to 75%, attained approximately 7 to 9 weeks after the operation.

For the past 3 years the total white cell count in over 100 normal uninfected rats of our colony has ranged from 14,000 to 24,000 per cu. mm. During the entire experiment the majority of the leucocyte counts in the control as well as the hypophysectomized groups fell within these extremes. Occasionally, in the hypophysectomized animals, the total white cell count showed an increase after operation which lasted for a few weeks, but this was also encountered in some of the control operated animals. There appeared to be no definite trend from week to week in any group. Individual counts in hypophysectomized animals sometimes revealed either an upward or a downward trend, and in general underwent greater fluctuation than individual counts in the control groups.

Differential counts made for all the hypophysectomized rats showed no significant variation from normal at any time after the operation.

Reticulocyte counts, made in 38 normal animals, ranged from 0.6 to 4.8%. Completely hypophysectomized animals almost invariably had a count of less than 0.1%, even as soon as 2 weeks after the operation, but occasionally this figure was exceeded. A reticulocyte count of less than 0.1% was also found in several of the operated controls. The reticulocytes of the starved normal animals showed a fluctuation similar to that observed in the operated controls, that is, 0.1 to 4.0%.

A study of the bone marrows of the hypophysectomized animals indicated a progressive hypoplasia. Within a month following the operation there occurred an increase in the number and size of the vacuoles throughout the length of the bone marrow. Between the 8th and 12th weeks, this hypoplasia was emphasized by the appearance of a somewhat centralized cavity extending throughout the length of the marrow. This cavity, as observed in sections, was filled with a clear coagulated liquid containing various amounts

Fig. 4. A SECTION THROUGH THE RIGHT FEMORAL BONE MARROW OF A NORMAL RAT. Bouin's; hematoxylin-eosin; 28×. Fig. 5. A SECTION THROUGH THE RIGHT FEMORAL BONE MARROW OF A RAT 3 MONTHS AFTER HYPOPHYSECTOMY. Bouin's; hematoxylin-eosin; 28×.

of cellular debris, or was completely devoid of tissue. There was also evidence of an increase in the number of fat cells in the remaining marrow tissue (fig. 4, 5).

DISCUSSION

The results of the present experiments indicate the occurrence of marrow hypoplasia and a true anemia, following hypophysectomy in the rat. Although a similar anemia has been claimed in the rabbit (1) the data accompanying this report are not conclusive, for the hemoglobin and erythrocyte counts both in the control and experimental animals showed too great an overlapping to be considered significant (20). Overbeek (4) failed to detect an anemia in hypophysectomized rats. The small number of animals which he used, however, along with the fact that the counts were made for only 3 weeks after hypophysectomy leave these results open to question. In a later work this investigator in collaboration with Querido (30) again assumed no anemia, yet an analysis of the data given in table 1 of that paper reveals lower red cell counts in the hypophysectomized than in normal animals, both before and during experimental treatment. Meyer et al. (14) concluded

that a red cell drop occurred after hypophysectomy, but failed to present direct experimental evidence for this anemia. The conclusion that a true anemia occurs, as indicated by the present work, is based on data from experiments designed to preclude objections made to previous investigations.

A direct relationship between the anterior pituitary and this anemia may be inferred from the report (15) on the presence of a hemotropic hormone in the hypophysis. The possibility, however, of any indirect connection between the pituitary and other structures must also be considered. Foremost among these are certain endocrine organs which atrophy after hypophysectomy. Kishi (21) and Kunde (7, 8, 9) have detected anemias in thyroidectomized animals. Hoskins and Sleeper (10) have found that thyroid therapy is successful in correcting the anemia of hypothyroid patients. The adrenal cortex is also alleged to have an influence on hematopoiesis (11) by virtue of the frequent occurrence of anemia in Addisonian patients. The possibility that the pituitary maintains the normal blood picture through either or both of these glands cannot at present be denied. The evidence in their favor, however, is not sufficiently conclusive to require acceptance. The anterior pituitary may also act upon the blood forming tissues through the action of other alleged principles of this gland. Perla (22) has found a spleen-stimulating factor in the anterior pituitary, and Wetzler-Ligeti and Wiesner (23) have obtained stimulation of the reticuloendothelial system with certain fractions of anterior lobe. Since the R-E system is intimately related with the hemolytopioetic mechanism, these 'restropic' factors may be involved.

It may not be necessary, however, to go so far afield for an explanation of the bone marrow aplasia and anemia. Granted that these follow hypophysectomy, other causes linked with known deficiencies that result from the operation might also be at work. The hypophysectomized rat, under optimal conditions, reaches a new level of existence. It loses 20 to 30% of its weight (19, 26), has a metabolic rate of about 70% of the normal (27), and digests and absorbs starch at a rate 30% below normal (28). One might refer to a 'seventy per cent homeostasis' which represents an adjustment to a complex of deficiencies arising directly and indirectly from hypophysectomy. This is seen clearly, in the present experiment, in the hemoglobin and erythrocyte plateaux eventually reached by the hypophysectomized rats. One factor in this general conditions, perhaps, is the lack of growth hormone, which Meyer and his coworkers (14) claim to stimulate reticulocyte formation in hypophysectomized rats. Another may be the lack of amino acids essential to blood building, for the hypophysectomized rats' protein metabolism is deranged and wasteful (26). Finally, as Sharp and Mack (18) point out, glandular dysfunction may lead to gastric disorders which in turn bring about protein depletion and poor absorption of minerals and vitamins.

The anemia and bone marrow hypoplasia following hypophysectomy in the rat may be interpreted in terms of effects on either blood formation or blood destruction. Dodds, Liu and Noble (24) report that posterior pituitary secretion stimulates blood destruction, and also according to Ponder (25)

estrin and estriol exert an influence on saponin hemolysis. More recently, Querido and Overbeek (30) have found that the reticulocyte response after injection of phenylhydrazine or bleedings is the same in both hypophysectomized and normal rats. From these results the authors conclude that the pituitary is concerned with the control of blood destruction rather than with blood formation. It is difficult to conceive how the anemia which develops after hypophysectomy can be explained on the basis of this hypothesis alone.

Although the results pertaining to the erythrocytes are quite conclusive, those dealing with the leucocytes are not. The white cell counts made on our animals seem to indicate that they are affected very little, if at all, by hypophysectomy. This is in agreement with the results given by Querido and Overbeek (29). Their counts, however, made on fresh bone marrow smears, indicate a fall in the relative number of lymphocytes. It might seem that the high total leucocyte counts sometimes obtained in operated animals, as reported in the present work, are due to the presence of infective organisms, to which hypophysectomized animals are especially susceptible. It has been shown, however, that these counts are within the limits of normal uninfected rats of the colony and, in addition, examination of the blood and tissues of the hypophysectomized animals reveals no trace of infective organisms. *Bartonella muris* may be excluded, since hypophysectomized rats treated with neo-salvarsan,[3] show the same fluctuations in white cell counts and decline in erythrocytes and hemoglobin as the other hypophysectomized animals.

The reticulopenia occurring after hypophysectomy has been given significance by Meyer et al. (14) who regard it as a certain indication of successful pituitary extirpation. Overbeek (4), however, finds a normal variation of reticulocytes ranging from 0 to 16% in peripheral blood and believes that only a change of a thousand per cent is of significance. The counts made in our animals tend to support this contention. Several of the control operated and normal starved rats exhibited extremely low reticulocyte percentages at various times. Furthermore, one of the completely hypophysectomized rats suddenly showed 5% reticulocytes after having maintained a 0.1% count for 6 weeks. Further investigation will be necessary before the significance of this post-hypophysectomy reticulopenia can be definitely stated.

SUMMARY

It has been shown that complete hypophysectomy in the rat is invariably followed by a decline in red cell count and hemoglobin, which attain approximately 60 to 70% their normal values after about 2 months. This anemia is not due to the lessened food intake or to effects of surgical procedure. No distinct trend has been found in the total or differential leucocyte counts of the animals; the counts of normal and hypophysectomized animals are subject to such wide fluctuation that no definite conclusion is warranted. The work of other investigators, showing that the hypophysectomized rat has a low circulating reticulocyte count, has been confirmed; but the reticulo-

[3] In our hands this drug has proved effective in arresting the Bartonella muris infection in splenectomized animals.

cyte counts in some controls at various times were also extremely low. The bone marrow of the hypophysectomized rat shows progressive hypoplasia. One might therefore conclude that the pituitary gland exercises a control over red blood cell formation, although some possible relation to blood destruction cannot, at the present time, be excluded.

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SPAWNING INDUCED PREMATURELY IN TROUT WITH THE AID OF PITUITARY GLANDS OF THE CARP[1]

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THIS PAPER deals with the problem of inducing rainbow trout to spawn prematurely under the influence of hormones contained in the pituitary gland of the carp and suggests endocrinological methods practical to fish culture. Certain investigations of this type have been made by other workers. Houssay (1) injected hypophyses of *Micropogon* and *Luciopimelodus* into the ovoviviparous fish *Cnesterodon* with the result that expulsion of eggs occurred 15 days before the voluntary spawning time.

In northeastern Brazil certain fishes spawn only after the heavy rains that follow a long period of drought. To procure eggs from these fish for artificial hatching purposes Cardoso (2) induced spawning prematurely in *Prochilodus* by injecting fish hypophyses. Von Ihering (3, 4) using this method, induced spawning prior to the rainy season (the normal spawning time) in *Prochilodus*, *Astyanax* and *Hoplias*. He secured expulsion of eggs within 24 to 96 hours following a single intramuscular injection of 1 to 5 hypophyses of these same species.

PROCEDURE AND RESULTS

Sexually mature rainbow trout (*Salmo gairdnerii*) and German brown trout (*Salmo fario*)[2] from the regular hatchery stock of the Madison Fish Hatchery were used in these experiments. The rainbow trout varied in standard length from 210 to 415 mm. (mean: 317) and the German brown trout from 180 to 313 mm. (mean: 238).

Whole pituitary glands were obtained from carp (*Cyprinus carpio*) which varied from 258 to 381 mm. standard length. During the months of October, November and December, 1938, the glands were collected, separated according to sex and stored in the frozen state. The mean weight of the pituitary gland of these carp was 8 mg. Some of the glands were desiccated with acetone to obtain a dry powder.

Equal amounts of male and female pituitaries were finely triturated and

[1] This investigation was aided in part through funds made available by the Wisconsin Alumni Research Foundation and the University of Wisconsin W.P.A. Natural Science Research Program.

[2] The facilities for these experiments and also the live carp and trout used in them were made available to us through the friendly cooperation of the Wisconsin Conservation Department. We acknowledge gratefully this cooperation.

suspended in 1 cc. of water regardless of the quantity of fresh or acetone dried glands injected. No fresh pituitary glands of the carp were over two weeks old when used.

All dosages of fresh glands are expressed in the tables in milligrams of fresh material while the acetone-dried glands are given in milligrams of dry material.

The pregnant mare serum used, had been stored in the frozen state and 0.3 cc., when injected in 21-day-old female rats over a period of 5 days, increased the ovarian weight from a mean of 13 mg. to 65 mg. The follicle stimulating hormone fraction administered in these experiments was prepared by a method based on the report of McShan and Meyer (5). The extract was made up so that 3.2 cc. were equivalent to 1 gm. of dried whole sheep pituitary powder. The injection of a quantity of this follicle stimulating extract equivalent to 500 mg. of dried sheep pituitary powder into 21-day-old female rats over a period of 5 days produced an increase in ovarian weight from a mean of 13 mg. to a mean of 60 mg. The ovaries of the rats were composed of follicles only.

Intraperitoneal injections of the gonadotropic extracts were given the fish in 1 cc. and 3.2 cc. doses. Injections were carried out in field unless the weather was too cold, in which case the fish were brought into a shed in tubs mounted on sleds. No fish were out of the water more than half a minute during injections.

As a regular part of the routine the temperature and oxygen content of the water were determined and recorded.

Since preliminary experiments showed that the male trout could be 'ripened' within a week (in November or December) with injections of 50 mg. of fresh pituitary glands of the carp, we injected males preceding each set of experiments to insure abundance of sperm for fertilizing any eggs procured.

Experiment 1. Into indoor brood house troughs supplied with running spring water, were introduced 42 female rainbow trout, about 15 to a trough. This group was injected with fresh carp pituitary glands in the amounts and at times indicated in table 1. A second group of 30 under the same conditions was injected with pregnant mare serum and a third group of 15 was not injected.

The results of these experiments are shown in table 1. Only 19% of the females spawned upon stripping one week after the initial injection of 25 mg. per fish of fresh pituitaries of the carp. By the second week 45% spawned upon stripping. Here after, the fish remained 'refractive' to further injections. Only one of the controls spawned up to the time of the close of the experiment on February 1, 1939. Two of the group injected with pregnant mare serum spawned during the injection period of 5 consecutive weeks. They were the only individuals of this group which spawned prior to February 1.

The fertilized eggs from the first spawning induced artificially were obtained on December 21, 1938. They hatched on February 1, 1939, one week before the regular hatchery spawning procedures began.

Experiment 2. This project was conducted in the outdoor raceways of the Madison Hatchery where more natural conditions could be simulated than in the confined

indoor troughs described in Experiment 1. Thirty female rainbow trout were introduced into each of 3 raceway partitions through which flowed a moderate current of spring water. One group of 28 received injections of fresh carp pituitary glands at weekly intervals. Another group of 27 was injected with acetone-desiccated pituitary glands of the carp and a third group of 30 was selected at random each week from a group of 500 uninjected females to serve as controls. The treated groups received injections in the amounts and at times indicated in table 2. Eight milligrams of acetone-dried carp pituitaries were the equivalent of 50 mg. of fresh material.

TABLE 1. EXPERIMENT 1

Date	No. fish	No. spawned	No. hard	Dose		Temp. degrees C.	Remarks
				Kind	Amount		
12-14-38	42	0	42	CP	25 mg.	9.0	
12-21-38	42	8	34	CP	50 mg.	8.9	
12-28-38	34	11	18	CP	50 mg.	7.8	5 died from fungus
1- 4-39	18	0	18	CP	50 mg.	8.2	2 removed for autopsy
1-13-39	16	0	16	CP	50 mg.	8.4	5 autopsied
1-20-39	11	0	11	CP	50 mg.	8.9	
2- 1-39	11	0	11				
12-14-38	14	0	14	PMS	1 cc.	9.0	
12-21-38	13	0	13	PMS	1 cc.	8.9	1 death
12-28-38	13	0	13	PMS	1 cc.	7.8	
1- 4-39	12	0	12	PMS	1 cc.	8.2	1 death
1-13-39	10	2	10	PMS	1 cc.	8.4	
1-20-39	10	0	10	PMS	1 cc.	8.9	
2- 1-39	10	0	10				
Controls:							
12-14-38	30	0	30	—	—	9.0	
12-21-38	28	0	28	—	—	8.9	2 deaths
12-28-38	28	0	28	—	—	7.8	
1- 4-39	27	0	27	—	—	8.2	1 death
1-13-39	27	0	27	—	—	8.1	
1-20-39	27	1	26	—	—	8.9	
2- 1-39	26	0	26	—	—	—	

CP: Pituitary glands of carp.
PMS: Pregnant mare serum.

A week after the first injection the fish were seined up and manipulated by experienced hatchery employees to test their readiness to spawn. If after easy stripping no eggs were obtained, the fish were reinjected and returned to the water to be examined a week later. The group treated with carp pituitaries responded more completely than those of Experiment 1. The stripping, one week after the initial injection, resulted in the successful spawning of only one fish. By the end of the third week, however, 95% of the injected females released all their eggs, in fact some had lost their eggs in the runway having spawned spontaneously during the third week. The 3 females that did not respond to the stripping were autopsied. No reason could be found for their failure to yield eggs upon manipulation.

Each week the 30 fish taken from the untreated hatchery stock were manipulated by stripping to test their readiness to spawn. It is noteworthy that none of these yielded to the stripping procedure until January 20, 1939, when one out of 30 spawned. On January 27, none of 30 trout could be stripped. When the hatchery spawning activities commenced on February 8, one out of 5 of the regular hatchery

stock spawned upon stripping. Rainbow trout from which this stock was taken were being spawned in the hatchery as late as March 22, 1939.

The fish in Experiment 1 responded better one week after the first injection than did those in Experiment 2. This discrepancy might be explained by the sharp drop in the temperature of the water in the raceways due to a cold wave during the week of December 23–30.

Experiment 3. This experiment was conducted concomitantly with Experiment 2. Thirty female rainbow trout were each injected with 3.2 cc. of follicle stimulating hormone and placed in an outdoor raceway partition similar to those used in Experiment 2. Another group of 30 trout each received 1 cc. of pregnant mare serum. The controls were those of Experiment 2. These mammalian preparations failed to induce

TABLE 2. EXPERIMENT 2

Date	No. fish	No. spawned	No. hard	Dose		Temp. degrees C.
				Kind	Amount	
12-23-38	27	0	27	dry CP	8 mg.[1]	7.8
12-30-38	27	0	27	dry CP	8 mg.	5.4
1- 4-39	27	10	17	dry CP	8 mg.	7.3
1-11-39	17	16	1	dry CP	—	7.7
12-23-38	28	0	28	fresh CP	50 mg.	7.8
12-30-38	28	1	27	fresh CP	50 mg.	5.4
1- 4-39	27	10	17	fresh CP	50 mg.	7.3
1-11-39	17	15	2	fresh CP	50 mg.	7.7
Controls:						
12-23-38	30	0	30	—	—	7.8
12-30-38	30	0	30	—	—	5.4
1- 4-39	30	0	30	—	—	7.3
1-11-39	30	0	30	—	—	7.4
1-20-39	30	1	29	—	—	7.8
1-27-39	30	0	30	—	—	7.6
2- 8-39	30	6	24	—	—	7.8

CP: Pituitary glands of carp.
[1] 8 mg. dry equivalent to 50 mg. fresh.

spawning in any of the trout after 4 weeks where each fish received one injection per week. Pregnant mare serum in 1 cc. injections failed to bring on spawning in 30 rainbow trout females after 1 injection during each of 4 consecutive weeks. There were no mortalities among these fish.

Experiment 4. To test the action of carp pituitary in hastening the ovulation time of German brown trout during their regular spawning period was the object of this experiment. Injections of 25 mg. per fish were made November 23, 1938 (toward the end of the spawning season of this variety in the Madison, Wisconsin, area) into 20 females that the hatchery men had selected as small and especially 'hard' trout. These fish were placed in troughs similar to those described in Experiment 1, and kept there for one week at the end of which they were stripped. The result was a 100% spawning. Out of 18 females that were not injected 6 spawned, and of the 19 fish injected with 1 cc. of pregnant mare serum, 13 spawned. The eggs were fertilized and developed normally.

The spawning period of the German brown trout lasts about 6 weeks, which necessitates a handling of the late spawners as many as 4 to 6 times which not only causes some mortality, but is a rather expensive procedure. It therefore appears

practicable to shorten the spawning season by hormonal stimulation. Other applications of this technic to fish culture are obvious.

DISCUSSION

The data submitted indicate definitely that the injections of suspensions of pituitary glands of carp have a hastening effect on the maturation and ovulation of the trout ovary, and that eggs can be procured, fertilized and developed normally at least 6 to 7 weeks in advance of the onset of normal spawning period. The experiments reported here were designed to meet the demands of fish culture procedure, consequently weekly injections were adhered to as a matter of practicability. The determination of the earliest time at which spawning can be made to occur by altering such procedures as quantity of pituitary injected and frequency of injections remains a project for future experimentation.

A gross comparison of trout ovaries in the spawning condition ('soft') with those in a pre-spawning condition ('hard') showed the latter to be more compact and opaque.

It should be emphasized that the acetone-dried carp pituitary glands were as effective in 'ripening' the ovary as were those stored in the frozen form. This method of storing the carp pituitary material is a more practical one and it is probable that the dried gland will retain its potency for long periods. At the present time we are investigating the possibility of using goldfish as a form for assaying the pituitary glands of carp which have been stored under different conditions and periods of time.

The reason for the unsuccessful results on trout that we obtained from pregnant mare serum and follicle stimulating hormone is not yet clear. Koch and Scheuring (6) also had no success with rainbow trout when they used a mammalian preparation (prolan), nor did von Ihering and Azevedo (4) induce spawning in *Prochilodus*, *Astyanax* and *Trachycorystes* with prolan, 'preloban' or fresh beef hypophyses. We hesitate, however, to conclude categorically that such a complete species specificity exists, for Calvet (7) and Damas (8) reported enlargement of the ovocytes and sexual maturity respectively prior to the normal development of the lamprey by subjecting the lampreys to baths of the urine of pregnant women (Calvet) and injections of ether-extracted pregnancy urine (Damas). Boucher, et al. (9) reported enlargement of the gonads of the eel after injections of the urine of pregnancy. Rugh (10) ovulated some of the Anura with antuitrin-S and whole sheep pituitary gland, and Mellish and Meyer (11) increased the weight of horned lizard's ovaries by injecting follicle stimulating and luteinizing fractions of the anterior pituitary of the hog in addition to pregnant mare serum. Furthermore, the influence of dosage, kinds of hormone, and season have been by no means exhaustively studied in relation to the spawning of fish. In our experiments we used chiefly follicle stimulating hormone preparations and it remains possible that a substance such as the luteinizing factor of the mammalian hypophysis, in the proper dosage, might be effective. There are probably other factors such as light and temperature which may be of

importance in influencing the action of hormones. Light may have been responsible for the difference in results found in Experiment 1 and Experiment 2, for Hoover and Hubbard (12) showed that controlled lighting influenced the development of the gonads of the brook trout (*Salvelinus fontinalis*). Other obvious factors that differed between the two groups were confinement and temperature. Experiment 1 was reported in this paper chiefly for the purpose of showing the variations possible in groups of fish of practically the same stock, but under different experimental conditions. Another difficulty with experimenting on trout in confined troughs was the mortality due to fungus infection. There were no mortalities in experiments conducted in the open air runways. Our experience leads us to believe that it is best to duplicate natural conditions as nearly as possible in studies of this type.

SUMMARY

Both mature eggs and sperm were obtained from rainbow trout 6 to 7 weeks in advance of the onset of the normal spawning period in Wisconsin by intraperitoneal injections of fresh or acetone-dried pituitary glands of the carp.

Pregnant mare serum and the follicle stimulating fraction prepared from sheep pituitary failed to induce spawning prematurely in trout.

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CASE REPORTS

CLINICAL EXPERIENCE WITH DIHYDROTACHYSTEROL IN THE MANAGEMENT OF IDIOPATHIC HYPOCALCEMIA

REPORT OF A CASE

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SINCE ITS INTRODUCTION in 1933 (1) considerable attention has been given the product dihydrotachysterol or A.T.10 (antitetanisches Präparat Nr.10). Holtz (2, 3) found it would relieve the tetany in parathyroidectomized animals and keep the blood calcium apparently normal, and the literature contains a number of articles attesting to its effectiveness in the treatment of hypocalcemia of hypoparathyroidism (4–28). The reports in the American literature dealing with the clinical use of dihydrotachysterol are still few in number. Hence, it was felt desirable to report our clinical experience with the preparation in the management of a case of idiopathic hypocalcemia, probably of hypoparathyroid origin.[2]

CASE REPORT

History. A 54-year-old Jewish housewife was admitted to the Jewish Hospital on November 28, 1938. Twelve years prior to admission she had experienced rather sudden spontaneous menopausal changes with which were associated few symptoms. However, complaints such as tiredness, weakness, 'dragging down' feeling and an inability to carry on as well as formerly dated from this time despite frequent medical attention. Three years prior to admission and 9 years after her menopause, she was left practically destitute by the death of her husband. Almost immediately following this she became very nervous and easily upset. She was at this time placed upon thyroid medication which she took for the next 2½ years. For a while she improved but soon began again to complain of the same tiredness and dragging-down feeling and, in addition, dyspnea while walking and a cramp-like pain in her arms and hands.

In July, 1938, while seated in the movies, there was experienced a sudden attack of cramps in the arms. She was able to leave her seat and reach the lobby by literally dragging herself. In the lobby she had a severe attack of spasm of her arms and legs. She described these parts as being extended, the wrists slightly flexed, the hands and fingers flexed rigidly. This spastic state lasted for a minute or two and then relaxed spontaneously. Since that time there have been several recurrences of a similar nature prior to her admission.

[1] From Medical Services of Dr. Joseph C. Doane and Dr. Mitchell Bernstein.

[2] The dihydrotachysterol was supplied by the Winthrop Chemical Company, Inc.

Physical examination. The patient was an elderly, white female in a good nutritional state, presenting a mild pallor and drooped facies, puffed face, thick lips and a general lethargic mien. Blood pressure was 130/80; temperature, 98.3° F.; pulse rate 80; respirations, 20. The skin was dry and scaly and the hair thin and dry. Bilateral, moderately advanced lenticular cataracts were present. No masses were felt in the neck. Results of examination of the heart and lungs and abdomen were negative. There was generalized hyporeflexia and a mild (Grade III) Chvostek sign. During the course of the examination there occurred another of the spontaneous attacks of spasticity which had been described. There was observed to occur, without obvious warning, a sudden generalized spasticity most marked in the extremities without associated tremors or convulsive movements. Her arms and legs were held in an extended position, the toes dorsiflexed, the wrists slightly flexed, the fingers rigidly flexed but not in a classical accoucheur position. The jaws were firmly clamped and a slight trismus was noted. This spontaneous attack lasted about one minute and was as sudden in its termination as it had been in its inception.

Laboratory findings. The results of repeated determinations of the hemoglobin, erythrocyte and leukocyte counts were within normal limits. Repeated urinalyses showed rather fixed low specific gravity with traces of albumin in all specimens and an average of 10 to 12 w.b.c. per high power field. B.M.R. readings of +10% and +15% were obtained on 2 occasions. Results of the Wassermann test were negative. The serum calcium was 5.7 mg.%, serum phosphorus 4.5 mg.%, total protein 7.95 gm.%, serum albumin 5.62 gm.%, serum globulin 2.33 gm.%, calcium ions, 2.4 mg.%, and the blood phosphatase was 8.12 Bodansky units. Results of various other blood chemical analyses were essentially normal.

X-ray of the bones revealed no areas of localized or generalized demineralization or decalcification. An electrocardiogram showed evidence of some mild myocardial degeneration and a left axis deviation.

Diagnosis. The essential physiological chemical changes that are typically found in cases of idiopathic hypoparathyroidism consist of: low serum calcium, high serum inorganic phosphorus and very low urinary calcium excretion. In the case being reported we were not equipped to accurately determine the urinary calcium excretion and the serum phosphorus was within the accepted limits of normal. However, McLean and Hastings (35) in their studies on the calcium ion concentration in the blood feel that, with the exception of hyperphosphatemia of nephritis and uremia, the finding of an abnormally low calcium ion concentration pointed to hypofunction of the parathyroid glands. Calcium ion concentrations below 4 mg.% constituted, in their opinion, abnormally low calcium ion levels. From their experimental work with the use of frog hearts, which are specifically sensitive to calcium in its ionized form, they were able to compute a chart giving the calcium ion concentration in blood in which total protein and total calcium concentration is known. The calcium ion concentration so determined in the case being reported was 2.4 mg.%. There being no hyperphosphatemia and no uremia or nephritis, we concluded, therefrom, that we were dealing with a case of hypocalcemia probably of hypoparathyroid origin. In the absence of such an etiological factor as a previous thyroidectomy we were unable to ascribe the cause of any hypofunction of the parathyroid glands.

Treatment. The initial therapy consisted of a high calcium-low phosphorus diet, calcium lactate by mouth, Vitamin D in the form of cod-liver oil and magnesium carbonate. Following this therapy A.T.10 was begun and the components of the initial therapy varied (fig. 1). During the course of treatment in the hospital the serum calcium levels were determined practically daily. In figure 1 is summarized

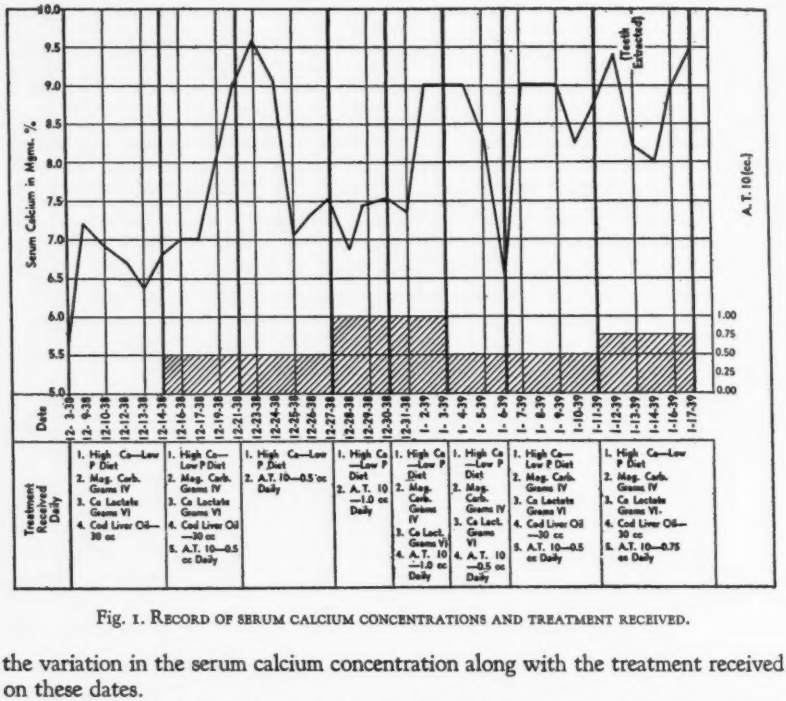
Serum Calcium in Mgms. %
A.T. 10 (cc.)
(Teeth Extracted)
Date
Treatment Received Daily
1. High Ca—Low P Diet
2. Mag. Carb. Grams IV
3. Ca Lactate Grams VI
4. Cod Liver Oil—30 cc
5. A.T. 10—0.5 cc Daily
1. High Ca—Low P Diet
2. A.T. 10—0.5 cc Daily
1. High Ca—Low P Diet
2. A.T. 10—1.0 cc Daily
1. High Ca—Low P Diet
2. Mag. Carb. Grams IV
3. Ca Lact. Grams VI
4. A.T. 10—1.0 cc Daily
1. High Ca—Low P Diet
2. Mag. Carb. Grams IV
3. Ca Lact. Grams VI
4. A.T. 10—0.5 cc Daily
1. High Ca—Low P Diet
2. Mag. Carb. Grams IV
3. Ca Lactate Grams VI
4. Cod Liver Oil—30 cc
5. A.T. 10—0.5 cc Daily
1. High Ca—Low P Diet
2. Mag. Carb. Grams IV
3. Ca Lactate Grams VI.
4. Cod Liver Oil—30 cc
5. A.T. 10—0.75 cc Daily

Fig. 1. Record of serum calcium concentrations and treatment received.

the variation in the serum calcium concentration along with the treatment received on these dates.

During the period of observation in the hospital, it was the rule for the patient to experience her previous symptoms of weakness, pains in the arms and legs and, on one or two occasions, repetitions of her spastic attacks when the serum calcium was at the low levels. On the dates when the serum calcium was maintained at the normal levels and at the time of discharge when the serum calcium was 9.5 mg.%, the patient admittedly felt stronger, better in spirit and free of complaints.

At the time of discharge (January 17, 1939) these concentrations were observed.

Serum calcium, 9.5 mg.%
Serum Phosphorus, 6.0 mg.%
Total proteins, 6.95 gm.%
Calcium ions, 4.30 mg.%
Blood phosphatase, 6.02 Bodansky units

She was taking at this time 0.75 cc. of A.T. 10 daily in addition to a high calcium-low phosphorus diet, calcium lactate, gr. XXX t.i.d., cod-liver oil oz. ½ b.i.d. and magnesium carbonate, gr. XX t.i.d.

Follow-up. Following her discharge from the hospital, the patient spent approximately 3 weeks at a convalescent home and a week at her own home before returning to the hospital for her first check-up. In table 1 the blood levels, the A.T.10 dosage and the symptom picture of the patient during the course of a fairly intensive follow-up period are given.

As noted in the hospital, whenever the serum calcium was maintained within normal limits, the patient remained essentially free of symptoms. Whenever the serum calcium was permitted to fall below the normal limits, the old symptoms of weakness,

TABLE 1. FOLLOW-UP STUDIES

Dates	A.T. 10 dose	Serum calcium	Serum phosphorus	Total protein	Calcium ions	Symptoms
1/17/39 to 2/2/39	cc. 0.75 daily	mg. % 9.2	mg. % 4.9	gm. % 7.35	mg. % 4.0	Asymptomatic
2/2/39 to 2/24/39	0.75 daily	—	—	—	—	Asymptomatic
2/24/39 to 3/3/39	0.	7.0	5.2	—	—	Buzzing in head—weakness
3/3/39 to 3/13/39	1.0 daily	8.0	4.5	—	—	No improvement
3/13/39 to 3/21/39	2.0 every other day	9.4	5.0	—	—	Much improved—stronger
3/21/39 to 3/24/39	2.0 every other day	10.5	4.25	—	—	Occasional slight buzzing in head—otherwise asympto matic
3/24/39 to 4/10/39	2.0 every third day	8.65	5.0	—	—	Some return of old weakness and ner-vousness—requested A.T. 10 every other rather than every third day

nervousness and a peculiar 'buzzing' in the head returned. In the last included follow-up period (March 24, 1939 to April 10, 1939), when an attempt was made to spread wider the interval between doses of A.T.10, it is interesting to note the request of the patient to return to the smaller dosage interval. Even though at this time the less frequent administration yielded a serum calcium concentration but slightly below the limits of normal and the symptomatic distress was mild, it was deemed best to restore and maintain a dosage of 2.0 cc. of A.T.10 every other day. Repeated examination of the eyes during this period revealed a slight progression in the lenticular cataracts.

DISCUSSION

Dihydrotachysterol or A.T.10 ($C_{28}H_{45}OH$) is a dihydro derivative of tachysterol which is one of the fractions obtained from the irradiation of ergosterol with ultraviolet light. The preparation is employed in an oily solvent containing 5 mg. per cc. which is stable indefinitely and adopted for peroral usage.

The initial and the maintenance dosage has been variously recommended (14). It must be appreciated, however, that no fixed schedule of dosage can be set that would apply to every case. The dosage must be completely an individual one varying with the needs and the response of the case. When the serum calcium has reached the normal level both the dosage and the frequency of administration may be reduced.

Many observers warn against the dangers of overdosage which are increased by the marked cumulative action of the drug. Excessive doses will produce hypercalcemia and severe toxic effects as manifested by anorexia, nausea, vomiting, headache, stupor, ataxia, thirst and skin eruptions. Large doses in animals have been reported to cause decalcification of the bones and metastatic calcification.

Since only small amounts of the drug are required and since excessive doses are harmful, the essential need for frequent determinations of the serum calcium concentration and complete individualization of the dosage of A.T.10 should be emphasized. To date no tolerance to the drug has been noted. Holtz (14) cites one patient who had been taking A.T.10 for 3 years without ill effect.

Jordans (25) and Holtz and Kramer (36) found that if a low calcium diet is used, calcium will be mobilized from the skeleton. On this basis, it appeared advisable to add calcium in excess of that contained in the usual diet. In addition to a high calcium-low phosphorous diet prescribed, we supplemented the A.T.10 therapy with calcium lactate by mouth (fig. 1).

In contradistinction to the pharmacologic action of parathyroid extract, the reaction of the body to dihydrotachysterol occurs slowly and persists for many days. MacBryde (4) mentions that with A.T.10 the first increase in serum calcium is noted in about 48 hours. The effect on the calcium persists for an equal period with a slight fall following its discontinuance. During the period December 27, 1939 to December 30, 1939 inclusive, when we increased the A.T.10 to 1.0 cc. daily as compared with 0.5 cc. daily previously, we failed to obtain any elevation in the serum calcium level (fig. 1). We feel now that the cause for this probably was the shortness of the observation period (72 hours). Had we maintained the same therapy and the same dosage a bit longer we possibly might have secured the slower acting effect of the dihydrotachysterol.

Many physiological variables may influence both the dosage and the effectiveness of A.T.10. Arnold, Holtz and Marx (37) found that 6 times as much A.T.10 was required toward the end of pregnancy as otherwise. During periods of marked activity (3, 21), nervous strains and menstruation, a larger dosage is usually necessary. The requirement for A.T.10 decreased in females after x-ray castration (24), and the action of A.T.10 was antagonized by estrin and the male sex hormone (37). During the last period of observation in the hospital (fig. 1), when the patient was apparently well stabilized on a dosage of 0.75 cc. of A.T.10 daily, there occurred a sudden drop in the serum calcium concentration. We ascribed this temporary lack of effectiveness of dihydrotachysterol to the fact that several teeth were extracted during this period. We felt that such a procedure probably involved several of the physiological variables just described as altering the effectiveness of A.T.10.

The lenticular cataracts so commonly seen in association with hypoparathyroidism can be possibly kept from developing by A.T.10, but are not influenced by the drug once they have formed (13, 14, 29, 30). It was noted

in this case that the lenticular cataracts found on admission seemed to have progressed slightly during the course of observation and follow-up despite control of the hypocalcemia with A.T.10.

The mechanism of the action of this sterol is not as yet definitely known although much work is being done in its study. Albright et al. (31) give an admirable summary of these studies to date. Holtz and Kramer (36) found that both fractions of calcium are elevated, the ionized and that bound to protein. In cases with tetany as opposed to normal controls, the effect is predominantly on the ionized fraction. With elevation in the serum calcium level there is an increase in the urinary calcium excretion (33) which may lead to stone formation (36). The fecal calcium excretion is decreased (32, 36). Hoff (34) found a slight elevation in the serum phosphorus level after treatment followed by a later fall. Others (9, 21) described a lowering of the serum phosphorus level following treatment. Halbertsma (22) mentioned a mobilization of calcium with an increase in the calcium level of the blood, but with no increase in the serum phosphorus level.

Albright and his associates (31, 38, 39) have made some fundamental and far-reaching investigations on the mode of action of A.T.10 comparing it with vitamin D and with the parathyroid hormone. They concluded that vitamin D and A.T.10 had the same two fundamental actions: first, to increase calcium absorption from the gastrointestinal tract and, second, to increase the phosphate excretion in the urine. The ratio of the calcium absorption factor to the phosphate excretion factor was less with A.T.10 than with vitamin D. The parathyroid hormone resembled A.T.10 as regards its property of producing a markedly increased urinary phosphate excretion but differed in that it probably had no primary action on calcium absorption from the gut. Their studies suggested that the biological action of A.T.10 differed from that of vitamin D in the direction of the parathyroid hormone. It seemed to them that if the difference could be somewhat further extended, one might actually synthesize a substance with the same biological properties as the parathyroid hormone, if not the hormone itself. If that would be the case, then the parathyroid hormone would be added to that large group of hormones whose chemical composition resembles cholesterol.

SUMMARY

A case is presented with an idiopathic hypocalcemia, probably of hypoparathyroid origin. No response was obtained with the common agents available in the treatment of hypocalcemia. The efficacy of dihydrotachysterol (A.T.10) as a therapeutic agent in the treatment of hypocalcemia is confirmed. The need for individualization of dosage of A.T.10 is demonstrated. A discussion of the action and the variables in the action of A.T.10 is presented.

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ANOREXIA NERVOSA

A CASE REPORT[1]

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SINCE 1872 and 1874, when Sir William Gull of Guy's Hospital described the syndrome of anorexia nervosa so accurately, little has been added to the original clinical description (1).

The clinical picture described by Simmonds (2) in 1914 in cases of destruction of the anterior lobe of the pituitary resembles in many respects the findings in anorexia nervosa and has given rise to considerable controversy regarding the etiology of anorexia nervosa as to whether it is of psychogenic (3, 4, 5, 6) or endocrine dysfunction (7). Many cases which are at first regarded as pituitary cachexia have proven on further investigation to be anorexia nervosa (8, 9). The following case demonstrates the similarities and differences in the two conditions.

The patient is a female, 21 years old, who was first seen in 1935 at which time she was somewhat underweight (height 66 inches, weight 113 pounds). There was no particular concern about her weight as her family were all tall and thin. She showed a low blood pressure, 95/68 and pulse 72. Mild acne was seen on face and trunk. Tuberculin test result was negative. Physical examination findings were otherwise negative.

She came in for treatment of the acne in November 1935 and was not seen again until June 1938 when she reported an amenorrhea since January of that year. On several occasions previously she had skipped one period, but never more than that. She had lost about ten pounds in weight. Time permitted only the following studies. Findings of x-ray of chest June 13, 1938 were negative for parenchymal disease. Basal metabolic rate was −18 and −19 with weight 90.64 pounds, height 66½ inches, pulse 60. Hemoglobin was 13 gm. Examination showed scaphoid abdomen with minimal amount of subcutaneous tissue, no masses or tenderness, external genitalia normal, normal distribution of hair, infantile uterus, no adnexal pathology.

In September 1938 she returned and reported that she had rested most of the summer and had eaten very well but had not gained in weight, nor had she had a menstrual period. Her weight on returning to school was 87 pounds.

The patient complained that she had a stomach ache if she ate very much. She had noted that if she rested for about an hour before meals she was able to eat more. She had, however, omitted breakfast as she experienced considerable epigastric discomfort when she had to hurry off to classes. This discomfort was described as a full feeling and a vague discomfort in the epigastrium which would last a variable length of time and which was not relieved by belching nor after a bowel movement.

[1] Presented before the Milwaukee Neuropsychiatric Association, Madison, Wisconsin, May 4, 1939.

The patient was moderately constipated. She also complained of nervousness and insomnia.

The basal metabolism test was repeated on September 29, 1938 at which time her weight was 87¾ pounds, pulse 48–68, B.M.R. −20, −24. Fasting blood sugar was 62. Sugar tolerance test showed fasting blood sugar 75 mg.%. One-half hour after ingestion of 50 gm. of glucose by mouth the blood sugar was 103 mg.% and at hourly intervals for the next 3 hours showed successively 167, 156 and 81 mg.%.

As it was suspected that possibly the patient was not eating as much as she reported, it was considered advisable to send her in to the Infirmary where her diet could be watched. Since she was unable to come in at the time, a food diary was kept which seemed to show good caloric intake for the two meals she ate. She was advised to arise in time to eat her breakfast slowly and rest before going to class. For one month she was on thyroid, one-half grain, with no appreciable result except continued loss of weight, though she was eating breakfasts with no apparent distressing effects. School examinations caused further loss of sleep and consequent further loss of weight to 86½ pounds. Vitamin B (9) extract was used with no appreciable effect.

In November 1938 the patient complained of very marked constipation and went as long as 5 and 6 days without a bowel movement. At times when she reported to the clinic she seemed to have plenty of energy but at other times she appeared tired but was able, nevertheless, to continue with her school work.

She entered the Infirmary November 5, 1938 for chloride restriction tests (10) and endometrial biopsy. She showed marked emaciation which was evident in her body and extremities. Breasts were not well developed but did not show any signs of atrophy. Hair was dry and the distribution was quite normal with no evidence of loss of hair in any region. Her skin was pale. No foci of infection were found, thyroid was not palpable, lungs normal, heart sounds faint, abdomen scaphoid; extremities were very thin, reflexes present but slow. Blood pressure ranged from 100/70 to 78/52. Pulse rate was very slow, 48 to 64. Her temperature was subnormal almost consistently, 96^6 to 98^4.

On the chloride restricted diet with high potassium (potassium citrate 1 gm. five times a day) she showed increasing asthenia but no critical nausea, emesis, back ache or prostration.

Date	*Time*	*Vol. of urine*	*% chloride*	*Total excretion*
11/27/38	7 P.M.–7 A.M.	1000 cc.	48 mg.	0.480 gm.
11/28/38	7 A.M.–12 Noon	450 cc.	94 mg.	.423 gm.

High salt with normal saline drinking water 1500 cc. daily, low potassium did not change the patient's condition.

She was given three 1 cc. doses of adrenal cortex (11) on the day before the endometrial biopsy and though this made her feel more energetic she did have some difficulty with accommodation and some vertigo. Attempts to obtain an endometrial biopsy on December 2 were unsuccessful. Previous observation of a small uterus was confirmed, the uterine cavity measured 6 cm. Both ovaries were small. No endometrium was obtained after several attempts with an endometrial punch, therefore a small curet was inserted into the uterine cavity but no endometrial tissue could be obtained.

X-ray of the skull showed no abnormalities of the sella.

She was given 1 cc. pituitary gonadotropic factor daily for 15 days. She had the injections for 3 days but discontinued them as she felt that they were responsible for

a peculiar attack of dizziness and light-headedness, and feeling of impending death which she had experienced the night following the third injection. The next day she felt so weak that she could hardly drag herself into the office. Findings were: temperature 97°, pulse 64, blood pressure 86/74, weight 85½ pounds. She had a slight cold in her head so was sent home to bed. She did not report for 4 days, then because of increasing weakness she returned to the Infirmary. She was found to have an infection in the left antrum which responded after several days' treatment. Emotional instability was very marked. The patient became progressively weaker, ate poorly and in general presented a very unfavorable picture. Five mg. of benzedrine sulfate was given which caused marked stimulation for several hours but was followed by a very marked let-down.

Daily injections of the pituitary extract were resumed for the remaining 6 days in the Infirmary, the last dose being given on December 19, 1938. An attempt was again made to determine whether there was a psychological background for her loss of weight. The only information was that the weight loss had started when she had restricted her diet in an effort to cure her acne. One food after another was eliminated from her diet. She felt that most foods except vegetables caused her epigastric distress and therefore they were omitted. After she had started to lose weight, more and more foods caused distress and were thus eliminated. She had always been a very poor sleeper and had a tendency to worry a great deal. She was a good student and took her work very seriously. She admitted a somewhat similar loss of weight but not as extreme at the age of 14, when there was a question of religious conflict which disturbed her considerably.

The patient was informed that she had no organic difficulty. She was persuaded that she merely needed food and that if she ate slowly she probably would have no distress. She cooperated well and ate everything on her tray, and also the supplementary feedings. After a few nights of rest she was considerably stronger. She gained 5¼ pounds in one week and this was all the proof necessary to convince her that she could gain weight if she would eat.

Repeated attempts to obtain vaginal aspiration specimens to determine if the gonadotropic factor was producing effects were unsuccessful. She weighed 98 pounds when she returned in January 1939, and felt much more energetic and cheerful. Her color and appearance were improved remarkably. She volunteered the information that she thought that most of the pains and aches she had complained of previously were largely mental. For the first time in months she joined in the activities with the other girls.

Constant encouragment has been necessary to continue to maintain weight gains. The patient again relapsed into her former habit of not eating breakfast. A normal menstrual period occurred January 28 to 31, February 18 to 22, March 12 to 16, April 20 to 24. Maximum weight attained 108¾ pounds. B.M.R. on February 13, 1939 was −8 and −13, weight 105½ pounds, height 67 inches, pulse 76-68 and 72-64. Constipation is somewhat improved. Insomnia is very little improved most of the time. Sugar tolerance test on March 8, 1939 showed fasting blood sugar 112 mg.%. Fifty gm. glucose was given by mouth and the blood sugar one-half hour later was 141 mg.%. One hour after ingestion of glucose the blood sugar was 156 mg.% and the next 2 hours 174 and 93 mg.%.

DISCUSSION

A brief review of this case shows an insidious onset of weight loss and associated amenorrhea (12) in a female of early adult age (13) who had had

a somewhat similar loss of weight at age 14, when religious conflict had been the underlying factor. She showed a definite psychoneurotic personality in addition to emotional instability, nervousness and insomnia. Mentally she was alert and bright. Vague gastro-intestinal (14) symptoms and constipation were prominent among her complaints.

Pertinent physical findings included slow pulse, low blood pressure, subnormal temperature, pallor and emaciation (15). Her physical energy and mental activity were in contrast to her emaciated appearance.

Laboratory findings included low basal metabolism (5), low fasting blood sugar (16), and indeterminate (17) sugar tolerance curve (18, 19, 20). Endometrial biopsy could not be obtained for lack of tissue. It was not possible to obtain a vaginal aspiration specimen for the same reason.

Her rapid and complete response to adequate diet with weight gain, restoration of normal activities, resumption of menses, increase in pulse rate, increase in blood pressure, disappearance of gastro-intestinal symptoms, elevation of B.M.R. and changes in sugar tolerance curve occurred only after the patient had been convinced that physically she was normal but that illness was due to her mental attitude.

Differential diagnosis. The usual wasting diseases were ruled out on absence of physical signs which would ordinarily be present before such a degree of weight loss would be manifest.

Simmonds' cachexia occurs predominantly in females though it may occur in males. The female patients are usually in the child bearing age (21–25), fourth and fifth decades most frequently, and the large majority of these patients have had multiple pregnancies; the onset of the disease often is related to a complicated pregnancy. It may occur when the anterior lobe of the pituitary is destroyed by tumor, granuloma or trauma. The emaciation is extreme. Atrophy of breasts, loss of hair, amenorrhea associated with loss of sexual function, loss of secondary sex characteristics differentiate Simmonds' disease from anorexia nervosa. The mental disturbance in Simmonds' disease may be very marked, even simulating Korsakoff's syndrome. A final differential point between the two diseases is the response to high caloric diet, which has no effect on Simmonds' disease.

Addison's disease (26) presents some of the symptoms of anorexia nervosa, loss of weight, hypotension, low fasting blood sugar, low basal metabolism. However, the use of the salt free diet in cases of Addison's disease shows characteristic changes in the blood chemistry, including lowering of the sodium and chloride and increase in the potassium level. Under standard conditions the concentration of sodium in excess of 293 mg. % in the 4-hour urine specimen on the morning of the third day of salt deprivation indicates abnormal adrenocortical function. Patients without adrenal insufficiency excrete from 44 mg. to 66 mg. per 100 cc. in the four-hour specimen (10).

Prognosis. The prognosis is good unless the diagnosis is postponed too long and death follows upon secondary infection in a patient with greatly lowered resistance (27, 28).

Treatment. Attempts should be made to determine the psychological

background of the condition and efforts made to readjust the patient's mental attitude toward eating. Once this is attained and the patient cooperates in taking a high caloric diet the recovery is remarkable.

Various glandular products, thyroid and anterior pituitary gonadotropic factor seemed to have no beneficial effect.

CONCLUSION

The extent to which the activity of all organs of the body is reduced when faced with chronic undernutrition is clearly demonstrated in this case. Of special interest is the inactive state of the endometrium as indicated by unsuccessful efforts to obtain biopsy specimen, which to my knowledge has not been previously reported in the literature. It is surprising that the mental activity withstands so well the ravages of undernutrition.

Thyroid extract and pituitary gonadotropic extract do not appear to have a beneficial effect in this condition.

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CLINICAL AND LABORATORY NOTES

A MODIFICATION OF THE ZONDEK ALCOHOL METHOD FOR DETERMINING BOTH LUTEINIZING AND FOLLICLE STIMULATING PRINCIPLES IN THE URINE

ROBERT T. FRANK*

IN 1935 A METHOD for determining both the leuteinizing and follicle stimulating principles in castrate and menopause urine was published from this laboratory.[1] Acetone was employed as a precipitant. The method was later employed in cyclical and amenorrheic women as well. In the latter groups the concentration of the gonadotropic principles is less and therefore unduly large amounts of urine were required, with frequent loss of animals from an undetermined toxic factor.

With the Zondek[2] alcohol method, smaller quantities of urine are required to obtain a follicle stimulating reaction, but the luteinizing reaction does not appear, especially if menopause urine is assayed.

During the last two years I have employed a modification of the alcohol method for both castrate and menopause urines as well as for cyclical and pregnancy urines in which smaller quantities can be used, which shows very low toxicity, and which demonstrates both the follicle stimulating and leuteinizing principles more consistently than by the acetone method.

Technic. (*a*) 400 cubic centimeters of fresh urine, or urine stored at 4.5° C. in the refrigerator is acidified with glacial acetic acid to PH 3.5 (Brom-Cresol-Green).

(*b*) Four volumes (1600 cc.) of 95% ethyl alcohol are added to the urine.

(*c*) Preparation is kept in refrigerator 6 hours to overnight at 4.5° C.

(*d*) Clear supernatant is poured off; remainder is centrifuged (250 cc. centrifuge tube).

(*e*) Precipitate is washed 3 times with ether, using 75 cc. for each washing. This removes all estrogen.[3]

(*f*) The precipitate may be dried under a fan or kept in refrigerator.

(*g*) Dry precipitate is transferred quantitatively into a 15 cc. centrifuge tube, using at most 6 cc. of distilled water for transfer.

(*h*) Preparation is thoroughly triturated with glass rod.

(*i*) It is centrifuged for 10 minutes; then supernatant poured into a graduated centrifuge tube and distilled water added to exactly the 6 cc. mark.

(*j*) The solution is now ready for injection into immature female rats (weight 30 gm.) without further PH adjustment (average PH 6.7—widest variations found 5.7 to 7.5).

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* From the Laboratories of the Mount Sinai Hosiptal, New York City.

[1] FRANK, R. T., U. J. SALMON AND R. FRIEDMAN: *Proc. Soc. Exper. Biol. & Med.* 32: 1666. 1935.

[2] ZONDEK, B.: *Klin. Wchnschr.* 9: 964. 1930.

[3] The gonadotropic extract representing 100 cc. of pregnancy urine has been injected into individual castrated mice without producing any change in the vaginal spreads. This demonstrates that estrogenic factors are removed completely by the ether washing.

In comparing the minimum amount of urine required to produce the same intensity of ovarian response with the acetone and the modified alcohol methods, it was found that in the menopause and amenorrhea urines only 1/3 the amount was required by the alcohol method. With pregnancy urine the same amounts of urine are found effective, irrespective as to whether acetone, modified alcohol or straight urine is employed.

Conclusions. A modification of the Zondek alcohol method is described for concentrating both the follicle stimulating and leuteinizing factors. For a complete bioassay of gonadotropic activity, this method is recommended.

I desire to acknowledge the assistance of Rose L. Berman, A.B., in this investigation.

NEWS ITEM

GRANTS FOR RESEARCH IN ENDOCRINOLOGY

IT IS ANNOUNCED that requests to the National Research Council Committee for Research in Endocrinology for aid during the fiscal period from September 1, 1940 to June 30, 1941 will be received until February 29, 1940. Application blanks may be obtained by addressing the Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington, D.C. In addition to a statement of the problem and research plan or program, the Committee desires information regarding the proposed method of attack, the institutional support of the investigation and the uses to be made of the sum requested. No part of any grant may be used by the recipient institution for administrative expenses. Applications for aid of endocrine research on problems of sex in the narrower sense cannot be given favorable consideration, but the Committee will consider support of studies on the effects of sex hormones on non-sexual functions—e.g., on metabolism.

CURRENT ENDOCRINE LITERATURE

COLLABORATORS

Fuller Albright
Wayne J. Atwell
Geoffrey Bourne
Israel Bram
John C. Burch
H. J. Deuel, Jr.
John C. Donaldson
Maurice H. Friedman
Murray B. Gordon
Authur Grollman
Frank A. Hartman
E. C. Hamblen
F. C. Koch
Edward Larson
J. T. Lewis
Joseph M. Looney
A. E. Meyer
Warren O. Nelson
J. P. Pratt
A. T. Rasmussen
Elmer L. Sevringhaus
L. B. Shpiner
D. L. Thomson
R. L. Zwemer

ADVANCE ABSTRACTS

American Physiological Society. Symposium on Carbohydrate Metabolism.

Cori, C. Glycogen breakdown and synthesis in animal tissues.
Soskin, S. The liver and carbohydrate metabolism.
Long, C. N. H. The adrenal and carbohydrate metabolism.
Young, F. G. The pituitary gland and carbohydrate metabolism. *To appear in this Journal.*

Boyd, E. M., and E. G. Mack. A method of assaying pituitary water retention principle. *To appear in this Journal.*

Existing methods of assaying the water retention principle of posterior pituitary extracts depend upon measurement of their renal antidiuretic activity. The authors worked out a method of assay based on retention of total body water (measured as uptake) of leopard frogs in water at 4° C. The low temperature permitted a more extensive and more accurate assay. The frog unit was based on the smallest amount of extract producing the maximal uptake of water in frogs under certain specified conditions.

Chiodi, H. The relationship between the thymus and the sexual organs. *To appear in this Journal.*

The thymus weight curve was studied in albino rats castrated before puberty (30 days) and in their corresponding controls. A real hypertrophy (controlled histologically) was found in the castrated animals, but the physiological involution was not retarded or modified. Adult castration was also found to produce a hypertrophy of the thymus. Injection of sex hormones (testosterone propionate or estrone) produced a marked atrophy of the thymus in normal or castrated rats. An Atrophy was also found after thyroidectomy in rats castrated before puberty. A slight hypertrophy of thymus was produced by adrenalectomy in rats castrated before puberty.

D'Amour, F. E. The potency of certain commercial hormone preparations. *To appear in this Journal.*

A total of 40 commercial preparations of the following hormones were assayed: estrogens, progestin, gonadotropins, growth hormone and cortin; the manufacturers' assay method being repeated. Most of the preparations studied contained the labelled potency, several did not. Certain specific findings were: (*a*) Aqueous solutions of estrin and progestin apparently deteriorate, this is also true of growth hormone preparations, (*b*) It is desirable that estrin preparations intended for oral administration be assayed orally; (*c*) The multiplicity of assay methods for gonadotropins has created a very confused situation, further complication resulting from the introduction of fractional units is considered undesirable.

D'Amour, F. E., and M. C. D'Amour. The biologic potency of International Standard Chorionic Gonadotropin. *To appear in this Journal.*

The biologic potency of the recently developed International Standard for Chorionic Gonadotropin (for pregnancy urine products) was determined by 5 assay methods: ovarian weight, luteinization, seminal vesicle weight, uterine weight and vaginal cornification. The authors believe that a combination of the last 2 methods provides a simple, objective and sensitive technic for the use of this standard in assaying pregnancy urine preparations.

Friedgood, H. B., and W. B. Cannon. Autonomic control of thyroid secretion. *To appear in this Journal*

In 2 of 28 cats a right phrenico-sympathetic anastomosis resulted in a syndrome originally described by Cannon, Binger and Fitz. Removal of the ipsilateral thyroid lobe in one cat was followed by a moderate but progressive decrease in the basal rate of metabolism; and subsequent resection of the anastomosis brought the metabolic rate down to a normal level. The cervical sympathetic trunks and the splanchnic innervation of the adrenals are not essential to the action of the anterior hypophyseal thyrotropic hormone. The question of sympathetic nervous system control of the thyroid function is discussed in detail.

Gray, S. W., and W. Ford. The effect of the crustacean eye-stalk hormone upon water metabolism and melanophore expansion in the frog. *To appear in this Journal.*

Color changes in the Crustacea have been shown to act on the melanophores due to a hormone elaborated in the eye-stalks. It has been demonstrated many times that either implantations of the pituitary gland or injections of extracts of the gland caused, in the frog, a marked dilatation of the melanophores and an increase in weight due to water inhibition. Therefore, it was of interest to find whether the eye-stalk of the crustacea had any similar influence when injected into the frog. To do this, a comparative study was made of the effects of *a*), pitressin (Parke, Davis); *b*), vertebrate pituitary bodies (fish, frog and turtle) and *c*), eye-stalks (Fiddler crab and crayfish) upon melanophore size and weight changes in the frog. The results show that in all cases there is both a melanophore expansion and weight increase in the frog following the injections. It was also demonstrated that quantitatively 1 cc. of pitressin had approximately the same influence as one vertebrate pituitary gland or 12 crustacean eye-stalks. Similar changes were demonstrated to take place in the hypophysectomized frog. These effects imply a similarity in the function between eye-stalk-hormone and the vertebrate pituitary.

Hechter, O., M. Lev and S. Soskin. The relation of hyperemia to the action of estrin. *To appear in this Journal.*

The experimental results described show that the effects of estrin may be divided into two groups, those specific actions of estrin which do not depend on hyperemia and those actions which are secondary to the production of hyperemia and which are associated with the acetylcholine liberating action of estrin.

Root, H. F., and C. Coggeshall. Acromegaly and diabetes mellitus. *To appear in this Journal.*

Twenty-nine cases of combined acromegaly and diabetes are reviewed. The average interval between the onset of acromegaly and that of diabetes was 9.2 years. A comparison of the symptoms in diabetes and acromegaly shows certain similarities. A constitutional or hereditary predisposition to diabetes underlying the diabetes encountered in acromegaly is suggested by the existence of hereditary or familial diabetes in 6 of the 29 cases, whereas among relatives of 127 acromegalics without known diabetes, only 2% had diabetes. Comparison was made of the weights of organs at autopsy between groups of patients with acromegaly, diabetes, combined acromegaly and diabetes and Simmonds' disease.

Seckel, H. P. G. The influence of various physiological substances on the glycogenolysis of surviving rat liver. *To appear in this Journal.*

With an in vitro method rat liver glycogenolysis, as it proceeds in surviving tissue slices suspended in a buffered salt solution, was found to be inhibited by large doses of cortical hormone. The degree of inhibition averaged about 41% at 60-minute intervals and with a concentration of 6 r.u. per cc. In female rats the results seem to be influenced by the hormonal tides of the sexual cycle. The inhibitory effect on hepatic glycogenolysis of cortical hormone is greater than that of insulin added in vitro and cannot be increased by combining the two hormones. If in vitro experiments year any relation to in vivo-physiology it may be concluded that one essential function of the cortical hormone consists in an inhibition of the glycogenolytic enzyme of the liver cells. The observations made in the living experimental animal are in good agreement with this conclusion.

UOTILA, U. U. The regulation of thyrotropic function by thyroxin after pituitary stalk section. *To appear in this Journal.*

An increase or decrease in an organism's thyroxin level acts on the anterior pituitary without any mediation or modifying action of the pituitary stalk. This is evidenced by the following findings. (*a*), Thyroxin given to male rats in daily 5 mg./kg. doses for 10 days causes a marked thyroid atrophy whether the pituitary stalk be severed or intact. Furthermore, other effects produced by thyroxin injection, such as weight loss, cytological changes in the anterior pituitary, adrenal cortical hyperplasia and atrophy of the seminal vesicles, are similar in stalk-cut or intact animals. (*b*), Residual thyroid tissue is still capable of compensatory hyperplasia after subtotal thyroidectomy even after the pituitary stalk has been destroyed. It is concluded that the thyrotropic function of the anterior hypophysis has a basic secretory rhythm which is for the most part controlled humorally by variations in the organism's thyroxin level without the mediation of the hypothalamo-hypophyseal pathways. Hypothalamic impulses through the pituitary stalk, or to a lesser extent the cervical sympathetics, can under certain conditions modify this basic thyrotropic secretory rhythm.

GONADS

PHILLIPS, W. A., AND L. E. YOUNG. Further studies on the rôle of progesterone in the inhibition of estrous cycles in the albino rat. *Am. J. Physiol.* 122: 175. 1938.

A method for preparing a crude extract containing estrogenic and progestational hormones from whole ovaries is described. It was found that the injection of an amount of extract containing 0.5 I.U. of progesterone and 0.1 I.U. of estrin and dissolved in sesame oil was sufficient to cause inhibition of estrus in mature female albino rats. The use of crystalline hormones (progesterone and estrin) in the same amounts had no inhibiting effect on the estrous cycles of the animals; at least 1.5 U of crystalline progesterone alone are necessary to produce this effect. The authors therefore believe their extract to contain a substance, not progesterone, which either enhances the effect of progesterone or which in itself is capable of inhibiting estrous cycles.—S. L. Cohen.

PINCUS, G., AND N. T. WERTHESSEN. The maintenance of embryo life in ovariectomized rabbits. *Am. J. Physiol.* 124: 484. 1938.

The effects of injections and implantations of progesterone and of 17-aethinyl testosterone, with or without simultaneous treatment with testosterone preparations, on the course of pregnancy were determined. Twenty-eight rabbits, ovariectomized 18 to 20 hours after mating were used. Examination of the uteri and ova of some of these animals, killed on the 5th day after mating, indicated that testosterone propionate, methyl testosterone and pregnandiol, when given subcutaneously in sufficient doses, each enhanced the effect of progesterone. It was also found that subcutaneous injection of at least 0.5 mg. progesterone was required to produce normal implantation during the first 11 days of pregnancy in the ovariectomized rabbits; a dosage of 1.0 mg. twice daily was required after the 11th day to maintain the embryos to term. Intramuscular implantation of 15 mg. progesterone maintained only a moderate degree of pseudopregnant proliferation, whereas similar treatment with 17-aethinyl testosterone was without effect. It is concluded that full gestation can be effected in ovariectomized rabbits by sufficient doses of progesterone alone, but that this effect may be enhanced by substances related to progesterone, and not themselves progestin. It is suggested that this enhancement is due to the prevention of the rapid destruction of progesterone in the body.—S. L. Cohen.

PLISKE, E. C. The follicular cycle in the sexually mature thirteen-lined ground squirrel (*Citellus tridecemlineatus* Mitch.) *J. Morphol.* 63: 263. 1938.

The germinal epithelium was studied in an effort to determine its rôle in postpubertal ovogenesis. Active and inactive areas of epithelium were observed regardless of the estrous periods. The active areas, exemplified by numerous mitoses and a frequent stratification of the epithelium, are foci for egg cell production. Egg cells are produced from the germinal epithelium by: activation, migration and transformation of single germinal epithelial cells; migration and differentiation of nests of cells; ingrowths of cords of cells. Young ova in the cortex of the ovary are at first surrounded by a single layer of flattened follicular cells of germinal epithelial origin. In the subsequent development of the follicle stratified layers make their appearance. The zona pellucida appears as a well-defined structure coincident with the stratification of the follicular layers. The follicular cavity develops as a split between the follicular cells at one side of the follicle. In the mature follicle the cavity is broad and contains liquor folliculi. The theca interna and externa are not distinguishable until the follicle is highly cellular.

POHLE, F. J. The blood platelet count in relation to the menstrual cycle in normal women. *Am. J. M. Sc.* 197: 40. 1939.

The average number of blood platelets in 13 normal women observed during a total of 82 menstrual cycles varied in a regular manner in relation to the menstrual cycle. This change was characterized by a slow progressive decrease during the 14 days prior to menstruation and a rapid increase soon after the onset of the menses. In individual cases there was usually either a slow progressive premenstrual decrease or a rapid decrease immediately before menstruation. In a few instances there was little or no cyclic change in the platelet and rarely a premenstrual increase occurred.—Author's Summary.

POWELL, T. C. Precocious hypertrophy of prostate following persistent treatment with gonadotropic hormone. *J. Urology* 41: 206. 1939.

A patient, 15 years of age, was treated with injections of APL substance following orchidopexy. The treatment covered about a year at which time marked interference with urination developed. This was due to prostatic hypertrophy, as shown by cystoscopic examination. Treatment was discontinued and the prostate gradually returned to normal size.—J. C. D.

RAYNAUD, A. Masculinization of female mouse embryos by injection of dehydroandrosterone in the pregnant female (Masculinisation des embryons femelles de souris par injection de déhydroandrostérone à la mère en gestation). *Compt. rend. Soc. de biol.* 129: 289. 1938.

Pregnant mice injected 5 to 9 days before parturition with 10 to 50 mg. of dehydroandrosterone gave birth to masculinized females and to males with stimulated male accessory sexual organs. Thus, dehydroandrosterone, a bisexual hormone by other criteria, proved to be only androgen in these experiments.—E. Cutuly.

RAYNAUD, A. Fate of the epididymis and Wolffian duct in intersexed female mice separated at birth from mothers injected with testosterone (Sur le sort de l'épididyme et du canal de Wolff chez quelques souris femelles intersexuées séparées dès la naissance de leur mère injectée a la testostérone). *Compt. rend. Soc. de biol.* 129: 528. 1938.

Two female mice were injected with 5 mg. of testosterone propionate 5 days before parturition. Their litters contained 4 and 3 females, respectively; these snimals were reared by lactating females which had received no treatment and were killed between the ages of 1 day and 3 months. Some of these females were intersexes, possessing epididymides, Wolffian ducts and male type urethras.—E. Cutuly.

RAYNAUD, A. Development of the male genital tract in intersexed female mice not receiving male hormone after birth (Evolution du tractus génital mâle chez les souris femelles intersexuées ne recevant pas d'injection d'hormone mâle après la naissance). Female genital organs in intersexed mice not receiving male hormone after birth (Étude de l'appareil génital femelles des souris intersexuées n'ayant pas reçu d'injection d'hormone mâle après la naissance). *Compt. rend. Soc. de biol.* 129: 632, 637. 1938.

Pregnant mice injected 3 to 8 days before parturition with testosterone propionate gave birth to intersexed females which were reared by normal foster mothers. Twenty such intersexes grew to adulthood, and data on these animals and on some younger ones form the basis of these reports. As late as 6 months after the birth of the intersexes it was possible to distinguish in them epididymides, seminal vesicles, prostate, Wolffian duct and male type urethra and external genitalia. The majority of intersexes had no nipples, but they had some mammary gland tissue. The x-zone of the adrenal was frequently present at 80 days, but tended to degenerate thereafter. Normal ovaries, tubes and uteri were present in the intersexed females; the lower portion of the genital tract, however, was modified.—E. Cutuly.

RIBOULLEAU, J. The amount of estrogenic substance in fertilized and unfertilized birds' eggs (Teneur en substances oestrogènes de l'oeuf vierge et de l'oeuf fécondé des oiseaux). *Compt. rend. Soc. de biol.* 129: 914. 1938.

Extracts were tested for their action on castrated rats and capons. The unfertilized egg extracts had no estrogenic power. Extracts from fertilized eggs showed an estrogenic effect equivalent to 1.5 to 2.0 γ of folliculin per gram of yolk. No masculinizing hormone was detected.—J. C. D.

ROUSE, S. B., AND E. W. BLANCHARD. The influence of calcium and potassium salts on the uterine contractions in normal and adrenalectomized rabbits. *Am. J. Physiol.* 123: 752. 1938.

Rhythmic uterine contractions were produced in ovariectomized rabbits, with or without adrenals, by the injection of a solution of theelin. The effect of slow intravenous injections of 2 cc. of 5% Ca or

KCl on these contractions were then observed, using a uterine fistula preparation. Both salts were found to produce sustained contractions of the uterus in both normal and adrenalectomized rabbits. These findings support the belief that Ca/K ratio alterations play no part in sensitizing the uterus to pituitrin at the time of parturition. The results also indicate that the adrenal cortex plays no significant rôle in the normal response of the rabbit uterus to the estrogenic hormone.—S. L. Cohen.

RUBINSTEIN, H. S., AND A. R. ABARBANEL. The use of testosterone propionate in the treatment of dysmenorrhea. *Am. J. Obst. & Gynec.* 37: 709. 1939.

Based on the concept that dysmenorrhea is due to relative excess of estrin and deficiency of progestin, the authors used testosterone therapy in 26 women with severe pain at menses. Doses were 5 mg. given 2 or more times preceding the expected flows. The were 16 with complete relief, 4 partially relieved, and 4 failed. Two had increased pain. Anatomic difficulties were commoner in the cases with poor responses. The authors attempt rationalization of the results on the basis of the physiological similarities of androgens and progestin.—E. L. S.

RUBINSTEIN, H. S., AND A. R. ABARBANEL. Effects of anterior pituitary extract on spermatogenesis in rat. *J. Urology* 41: 726. 1939.

Descent of the testis occurs at about 45 days in rats. Ten males under this age and 10 older received in the course of 10 days, 160 R.U. of alcoholic extract of sheep anterior lobe. They were compared with litter-mate controls. The authors conclude: "In the normal immature male rat, anterior pituitary sex hormone (in the dosage used) causes premature descent of the testes without increase in weight. It also stimulates the primary germ cell line to markedly active proliferation. The stimulated cells which may be found cast off in clumps within the epididymis show no evidence of hastened maturation. Interstitial tissue is increased and precocious development of the epididymis to adult proportions and characteristics results. In the normal mature rat, anterior pituitary sex hormone causes a definite increase in the weight of the testis, a moderate increase in proliferation of the germinal epithelium and a moderate widening of tubular diameter."—J. C. D.

RUBINSTEIN, H., AND A. A. KURLAND. The effect of testosterone propionate on spermatogenesis in the human. *South. M. J.* 32: 499. 1939.

In 6 normally constituted males testosterone, 5 mg. intramuscularly 3 times a week, increased the sperm count. In another case with large numbers of immotile sperm, the relative number of motile sperm was increased. One case did not respond and was found to have obstruction of both vasa. Larger doses, 25 mg., had a depressing effect on both sperm motility and libido, which was stimulated by the smaller amounts. There was no effect on the external genitalia. After treatment was stopped, all patients reverted to their original condition.—J. C. D.

SALMON, U. J., S. H. GEIST AND R. I. WALTER. The treatment of dysmenorrhea with testosterone propionate. *Am. J. Obst. & Gynec.* 38: 264. 1939.

In 30 women between ages 15 and 45, with dysmenorrhea the treatment was 10 to 50 mg. testosterone propionate in oil 3 times weekly, for various periods from 1 to 4 weeks per cycle. Total doses varied from 100 to 1500 mg. Success is reported in 26 women. It is evident that the larger doses inhibited estrogenic activity at times, which effect was temporary. In some cases changes in voice, hair pattern and clitoris size followed large doses, but never with doses as small as 300 mg. total. The theory of the authors is that the normal ovary produces androgenic material, and that dysmenorrhea results from a deficiency of this material.—E. L. S.

SAMUELS, J. An exact method of determining ovulation and pregnancy. *Surg., Gynec. & Obst.* 67: 608. 1938.

The author describes a spectroscopic method of ascertaining the daily reduction figures of oxyhemoglobin by means of an instrument called the cycloscope or spectroreductometer. With this instrument it had been determined that in healthy normal people with a balanced endocrine system, the reduction time is 150 seconds. With men and women in the climacteric the reduction figures taken daily are 145 seconds. In 20 sexually mature women between the ages of 25 and 40 years all ovulated 2 times monthly.

SCHACHTER, B., AND G. F. MARRIAN. The isolation of estrone sulfate from the urine of pregnant mares. *J. Biol. Chem.* 126: 663. 1938.

The free and conjugated chromogenic estrogens were extracted from pregnant mare urine with butanol, washed with 1 N NaOH and then acidified and extracted with ether to remove ether-soluble

impurities. This water-soluble material was again extracted with butanol and evaporated to dryness. The residue was taken up in chloroform to which a small amount of ethanol was added and the conjugated chromogenic estrogens extracted with water. The water-soluble material was converted to the barium salt and dissolved in butanol and then washed with normal KOH and evaporated to dryness. This material was heated with 98% acetone and the insoluble residue discarded. The acetone was distilled off and the residue again extracted with acetone containing a few drops of water, and the solution concentrated until crystals appeared. The aqueous-acetone solution was evaporated to dryness and the residue recrystallized from alcohol-ether mixture. Analysis of the material showed it to be the K salt of estrone sulfate.—J. M. L.

Schäfer, G., and H. Meister. Progynon in management of mastitis (Progynon in der Behandlung der Mastitis). *Zentrlbl. f. Gynäk.* 63: 796. 1939.

Making use of the known inhibition of lactation by estrogens, the authors attempted to secure a temporary and partial inhibition in women with febrile course and painful, caked breasts during early lactation. In 28 cases only 3 came to incision, as compared with 26% in the authors' earlier series with conventional treatment. The relief was prompt, and the average clinical course was reduced from 18 to about 10 days. Lactation was not permanently stopped, infant weight gain continued. The doses were usually 50,000 international benzoate units, given on 1 to 3 days.—E. L. S.

Schapft, E. The action of estrogen injected into the uterus (Ueber die Wirkung intrauterin injizierten Follikelhormons). *Zentrlbl. f. Gynäk.* 63: 788. 1939.

Based on earlier animal work the author tried injecting 5000 I.U. of estradiol benzoate into the anterior lip of the cervix in cases of amenorrhea and sterility, the total dose in one series or during one menstrual cycle being not over 30,000 U. Of 13 cases there was evidence of benefit in 11. Pregnancies occurred in several cases. The doses appear to be smaller in proportion to the results than when the material is injected into skeletal muscles.—E. L. S.

Schmidt, I. G. The influence of a normal ovary on the formation of typical irradiation tissues in the x-rayed ovary. *Anat. Rec.* 71: 409. 1938.

When both ovaries of sexually mature guinea pigs were irradiated with a minimum sterilization dose of x-rays, the normal ovarian stroma was entirely replaced by glandular cells formed from follicles of all sizes. Accompanying this change were irregular estrous cycles with prolonged estrous openings. One ovary of a pair, in 12 animals, was similarly irradiated to observe the effect of the glandular tissue on the functioning of the untreated ovary. The expected results were reversed, for the presence of the normal ovaries prevented the formation of the glandular tissue in the irradiated ovaries. The latter were atrophic and devoid of follicles. Estrous cycles were not interrupted by the x-ray treatment and continued to be regular and normal, maintained apparently by the normal untreated ovaries alone.—Wistar Bib. Service.

Schneider, P. F., Prophylactic endocrine therapy in artificial menopause. *Am. J. Obst. & Gynec.* 37: 860. 1939.

Based on observations on 519 women treated for various disturbances of climacteric syndrome type, the author points out the value of the use of adequate doses and early institution of the use of estrogens to secure relief. He uses intramuscular injection to determine the required dose, then transfers to use of amniotin, progynon, or emmenin orally employed these materials in this order for descending doses. The use of vaginal smears serves to demonstrate the results in some cases. He feels that preoperative use of estrogens before ablation of ovaries is helpful.—E. L. S.

Schnidt, K. Concerning quantitative prolan estimations in toxemias of pregnancy (Ueber quantitative Prolanbestimmungen bei Schwangerschafts-Toxikosen). *Ztschr. f. Geburtsch. u. Gynäk.* 116: 56. 1937.

In a comparison of quantitative urinary prolan estimations in normal pregnancies and toxemias of pregnancies, there was frequently an increase in the prolan in the toxemias. In 35 toxic patients there was an increase of 70% in prolan A and 25 to 40% in prolan B. Of 15 normal pregnancies in the different months of gestation, there was an increase in prolan in only one. This patient had a twin pregnancy. No practical application was suggested.—Milo Ritter White.

Schrick, E. W. Insulin treatment of dysmenorrhea. *Am. J. Obst. & Gynec.* 37: 146. 1939.

Ten unmarried students, ages 16 to 29, whose menstrual periods were regular and normal except for pain of varying degrees of severity were given Squibb's regular insulin, 5 units daily before lunch for

3 to 5 days before each menstrual period for 2 or 3 months. Eight patients were benefited while under treatment, and 3 had several menstrual periods free from pain without the use of insulin. The cramplike pains of dysmenorrhea have been relieved by a single injection of Squibb's regular insulin 5 to 7 units, with a supply of carbohydrate. Several workers have suggested that insulin may work through its hypoglycemic effect, but efforts to control the antidiabetogenic effect of insulin and a few blood sugar studies would seem to discredit this hypothesis. At present it appears that insulin therapy in functional dysmenorrhea is successful because of its direct effect on the ovaries or hormonal blood balance.—E. L. S.

Schürger, S. Can medical induction of labor be improved with estrin? (Lässt sich die medikamentöse Geburtseinleitung mittels Follikelhormon verbessern?) *Zentrlbl. f. Gynäk.* 63: 207. 1939.

In 18 cases with labor absent after term the induction was by use of castor oil plus 10,000 or 50,000 units of estrin (? route), followed by 2 to 8 doses of 0.2 to 0.3 cc. thymophysin. In 15 cases birth followed in 5 to 16 hours, without difficulties of unusual type.—E. L. S.

Spencer, A. W., and E. S. Scowen. Hormone treatment of imperfect descent of the testes. *Lancet* 235: 983. 1938.

The author reports on 65 patients varying between the ages of 5 to 26 years. Of 33 with bilateral maldescent 28 were of the retractile type and in 32 with unilateral descent 5 were of the retractile type. The doses used were not less than 500 U twice weekly. In successful cases the treatment was continued for 1 to 2 months after descent had been effected, to insure against subsequent retraction. All patients were seen at 3 and 6 monthly intervals after treatment was completed. It is the authors' opinion that degeneration of the testes does not follow the use of homologous extracts, therefore the treatment in selected cases may be continued for some length of time. If the treatment is unsuccessful after 18 months the condition may be due to an anatomical anomaly. In the authors' experience the successful result appeared more likely in bilateral (69%) than in the unilateral (56%).—L. B. S.

Stein, I. F. Further studies in infertility and sterility. An analysis of 200 couples. *Surg., Gynec. & Obst.* 67: 731. 1938.

Of the 200 couples studied, infertility was more often encountered than sterility, the male partner being responsible for ⅓ of the failures. In the infertility group, pregnancy occurred in 50% following the correction of the factors constituting the barrier to conception. Successful treatment in this group was chiefly attributable to cervical cauterization, potency tests and sex adjustments. In the sterility group (inability to conceive) 15.4% became pregnant after investigation and treatment. The successful correctives in this group consisted of hormone therapy in the male and surgical treatment in the female.—L. B. S.

Stemmer, W. Synthetic proluton (Ueber synthetisches Proluton). *Zentralbl. f. Gynäk.* 61: 2592. 1937.

Satisfactory results are reported in the following types of ailments: 1. Bleeding in metropathia glandularis cystica, 8 cases treated with doses of 2 to 5 mg. daily. 2. Elimination of lumps in menstruation, considered as a sign of insufficient formation of enzymes in the uterus; two cases treated with 2 times 5 mg. per week. 3. Certain types of dysmenorrhea, treated with 5 mg. twice weekly; two cases reported. 4. Imminent abortion, treated with 2 mg. every 2 or 3 days; the treatment was successful in 4 cases, one patient aborted when the medication was discontinued, 2 cases did not respond. In some cases cortin was also used. 5. Toxicosis in pregnancy was treated concomitantly with cortin. The results were not sufficiently definite as to permit any conclusions. 6. Amenorrhea caused by deficient luteinization was treated with progynon and proluton, the latter given in doses of 2 times 5 and 3 times 2 mg. The 7 cases treated responded with bleeding of variable length and intensity. 7. The prevention of lactation requires large doses. Positive results were obtained in 50% of the cases treated.—A. E. M.

Tapella, P. A. Effect of estradiol on compensatory hypertrophy of the ovary (Action de l'oestradiol sur l'hypertrophie compensatrice de l'ovaire). *Compt. rend. Soc. de biol.* 128: 1132. 1938.

One whole ovary and one-half of another ovary were removed from 66 rats. Some of these animals served as controls and others received over a period of 6 weeks daily doses of estradiol benzoate ranging from 2.5 I.U. to 500 I.U. The small doses of hormone inhibited compensatory hypertrophy of the ovarian fragment, and the larger doses caused atrophy of the remnant.—E. Cutuly.

Takata, M., On the determination of the day of ovulation in woman by means of a quantitative flocculation of the serum (Zur Ermittlung des Ovulationstages bei der Frau mit Hilfe der "Flockungszahl reaktion" im Serum). *Arch. F. Gynäk.* 167: 204. 1938.

The test which the author used was a type of quantitative flocculation test, using the Takata reagent, on the serum. He carried out these tests on 57 women, doing a total of 1,537 determinations. Of the 57 women 47 had normal menstrual cycles. The daily variations in the test in 102 individual cycles were studied. It was found that if the results were plotted in a curve there was a depression lasting

several days at the time of menstruation and a sharp depression lasting 1 day in the mid-interval. This sharp depression the author interpreted as being due to ovulation. He found on this basis that the day of ovulation had a constant relationship in any one individual with the previous menstruation, rather than with the following menstruation as the teachings of Igino and Knaus have led us to believe. He also followed one patient throughout pregnancy, but found no fluctuations until delivery when there was a sharp rise followed by a fall. Menopausal and amenorrheic patients showed no fluctuations. The author believes that the curves show the amount of estrogens in the blood and found that estrin in vitro decreased the flocculation. He obtained corresponding results by estrin or progestin injections. —F. A.

TAYLOR, H. C., AND E. N. SCADRON. Hormone factors in the toxemias of pregnancy, with special reference to quantitative abnormalities of prolan and estrogens in the blood and urine. *Am. J. Obst. & Gynec.* 37: 963. 1939.

Assays for prolan and estrogen were made by methods modified from those in conventional use, in 21 cases of late toxemia and 17 normal pregnancies. There is a tendency to higher prolan values in toxic cases, and of lower estrogens, and in a few cases of lower pregnandiol excretion. Results are not uniform enough to justify conclusions, nor to indicate whether the changes are the cause of toxemias, or the results of the toxic states.—E. L. S.

TENNEY, B., AND F. PARKER, JR. The significance of the weakly positive Aschheim-Zondek test. *New England J. Med.* 218: 561. 1938.

A study was made of 60 cases showing a weakly positive A-Z test. Of these 38 resulted in miscarriages; 12 were tubal pregnancies, 5 had dead fetuses, 2 hyperplasia of the endometrium, 2 showed tubo-ovarian masses and 1 was a hydatid mole. None of these cases resulted in a normal pregnancy and the finding of a weakly positive test is therefore indicative of a poor prognosis.—J. M. L.

THOMPSON, W. O., AND N. J. HECKEL. Undescended testes. Present status of glandular treatment. *J.A.M.A.* 112: 397. 1939.

With 38 patients of all ages, descent was produced in only 10 of 50 undescended testes, or in 20% compared with an average of 61% of successful results reported in the literature. If only the 28 patients under 16 years of age are considered, descent was produced in 9 of 33 undescended testes, or in 27%. Descent did not occur in any instance in which the testis was intra-abdominal or deflected over the external oblique muscle.—From the Authors' Abstract.

THOMPSON, W. O., N. J. HECKEL, AND A. D. BEVAN. Influence of anterior pituitary-like principle on external genitalia of young boys. *J. Urology* 40: 145. 1938.

This hormone was tested on 28 patients with undescended testes. In those under 12 years of age, actual descent occurred in 33%. These were cases where the testis was well down in the canal. Anatomical obstructions explained failures in others of this age group. Successful results occurred within 9 weeks. Since growth of the genitalia and prostate is produced by prolonged treatment, it should be discontinued after 2 months, if not successful. Even where actual descent of the testis was not achieved, the treatment facilitated surgical procedure by enlarging the parts involved.—J. C. D.

THOMPSON, W. O., AND N. J. HECKEL. Variations in genital and somatic development wih bilateral cryptorchidism. *Proc. Central Soc. Clin. Research, J.A.M.A.* 112: 1105. 1939.

A study has been made of 11 males from 15 to 29 years of age with bilateral cryptorchidism. In no instance was somatic development completely normal. When both testes remained within the abdominal cavity there commonly resulted infantile genitalia, a high-pitched voice, long extremities, broad hips and absence of hair on the face and body. This was not uniform, as demonstrated by a normal-size penis in two cases in which neither testis could be palpated. In one patient with bilateral intra-abdominal testes genital growth was produced with APL principle. Usually, however, growth could not be produced in such instances. Spermatogenesis was not observed in any patient, either before or during treatment with the APL principle. Testes in the inguinal canal are more capable of function than those in the abdomen, as demonstrated by frequent growth of the genitalia in such instances, with and without administration of the APL principle. Both testes can be completely normal only when they are in the scrotum. However, the interstitial cells may show a surprising degree of function even though both testes remain within the abdomen.—Abstract from *J.A.M.A.*

TYNDALE, H. H., L. LEVIN AND P. E. SMITH. Responses of normal and hypophysectomized immature rats to menopause urine injections. *Am. J. Physiol.* 124: 174. 1938.

The effects of injections of menopause (CU) and of pregnancy urine (PU) extracts on the development of corpora lutea and on the ovarian weights of 349 normal and 77 hypophysectomized rats were

studied. While from 8 to 10 times as much CU extract was required to produce luteinization in hypophysectomized rats as in normal animals, the occurrence and degree of luteinization in the former group was much more consistent with the dosage of CU. It is therefore suggested that the luteinizing principle in such extracts acts most efficiently through the pituitary, but the pituitaries of the normal animals may interfere with the responses. The ovarian weight responses also showed a much greater degree of individual variation in the normal than in the hypophysectomized rats, but the dosages of CU required here were more closely related for the 2 groups of animals than for the luteinization response. PU extracts differ from CU extracts in that the former produces both increased ovarian weight and luteinization in normal rats in low dosage, while the CU extracts produce large increases in ovarian weight before luteinization begins. Because of the large variations in responses the authors believe that quantitative assays of luteinizing and follicle-stimulating hormone in PU and CU extracts are of questionable value.—S. L. Cohen.

VARGAS, L., AND A. LIPSCHÜTZ. Origin of connective tissue tumors produced by estradiol benzoate (Sur la genèse des tumeurs conjonctives provoquées par le benzoate d'oestradiol). *Compt. rend. Soc. de biol.* 129: 810. 1939.

Ten castrated female guinea pigs received 80γ of estradiol subcutaneously 3 times a week. Theywere killed after 2 to 4 months of treatment. After 2 months tumors appeared and were present eventually in all animals. Two had uterine-tubal tumors only, 2 had tumors outside the genital tract, and 6 had tumors in both locations. The extra genital tumors appeared first. The tumor location was unrelated to the area of surgical interference or of injection.—J. C. D.

VENKATACHALAM, K., AND A. N. RATNAGIRISWARAN. Efficacy of gonadotropic hormones in treatment of whooping cough. *Indian M. Gaz.* 74: 27. 1939.

On the ground that whooping cough is a disease that shows a preference for attacking children less than 10 years of age, and old persons occasionally, and that adults are invariably immune to it the authors considered it probable that the power to resist and overcome the disease might be closely associated with the activity of the sex glands, the dominant feature of adult life. Based on this assumption, they used a charcoal adsorbate of the gonadotropic substance of pregnancy urine (orally or parenterally) in the treatment of 136 children. Satisfactory results were obtained in 80.3% of the cases. The most severe symptoms (whooping, vomiting and coughing) were alleviated in about one week. The drug was also found to be effective as a prophylactic, in that it conferred an immunity.—From abstract, *J. A. M. A.*

VENNING, E. H. Further studies on the estimation of small amounts of sodium prenandiol glucuronidate in urine. *J. Biol. Chem.* 126: 595. 1938.

Some improvements and refinements in the method for isolating sodium pregnandiol glucuronidate from urine are described. Urine samples should be kept at 4°–7° C. to prevent hydrolysis of the compound to free pregnandiol and subsequent loss. Vigorous shaking should be avoided during extraction with butyl alcohol as the formation of emulsions caused loss of the pregnandiol compound. The amount of water added to the acetone for reprecipitation of the compound must be varied from 2 cc. if less than 5 mg. of glucuronidate was present, to 5 cc. for 10 mg. or more. Enough urine must be taken to yield 4–5 mg of sodium pregnandiol glucuronidate in order to obtain reliable results.—J. M. L.

VEST, S. A., AND J. E. HOWARD. Clinical experiments with the use of male sex hormones: Use of testosterone propionate in hypogonadism. *J. Urology* 40: 154. 1938.

This hormone was used in 6 cases of adult hypogonadism, and in 2 boys to hasten puberty. These cases are reported in full. The anatomical, physiological and psychological changes indicated that this was satisfactory as a replacement therapy.—J. C. D.

VILLELA, G. G. Determination of androgenic hormones by the chicken's test (Dosagem dos hormonios masculinos pelo "test" do pinto). *O Hospital* 15: 489. 1939.

One-day-old Leghorn chicks are suitable for androgenic hormone determinations. The chick comb test proposed by Dorfman and Greulich is easier to carry out than is the capon comb test and shows greater sensibility. The lower price of chicks and their lower cost of upkeep permit the use of large numbers economically. The determinations reported showed that the ratio between the comb's area (determined by planimetry) and body weight may be employed together with that of Dorfman and Greulich to confirm clearly their results. The ratio comb area: body weight showed constant values for both sexes.—Author's Summary.

VLCEK, J. The estrous cycle in avitaminosis (Le cycle oestral dans l'avitaminose). *Compt. rend. Soc. de biol.* 129: 114. 1938.

Vitamin E deficiency in rats caused a lengthening of the interval between periods of estrus. When

the deficiency did not prevent delivery in pregnant animals, reappearance of estrus depended on the survival of the young; if the litter died, estrus occurred in a few days, otherwise it was inhibited as long as the litter suckled. Follicular hormone injected into E deficient rats induced short and frequent periods of estrus; prolan seemed not to have any effect on the cycle.—E. Cutuly.

Volavsek, W. Hormone treatment of vulvovaginitis gonorrhoica (Zur Hormonbehandlung der Vulvovaginitis gonorrhoica). *Dermat. Wchnschr.* 106: 410. 1938.

The treatment consisted in an initial intramuscular injection of 10,000 U of estrin followed by 4,000 U daily, given orally, until the smears became negative. Oral treatment with 3,000 U daily was continued for three weeks. Concomitantly, local treatment was given. The 12 cases treated required at an average 26.5 days until the gonococci disappeared. No recurrence was observed during observation for over 6 months.—A. E. M.

v. Pallos, K. Modified Fluhmann procedures Uefulness and evaluation of quantitative estimation of liquids containing small amounts of estrin (Modifiziertes Fluhmann Verfahren. Brauchbarkeit und Verwertung zur quantitativen Bestimmung von wenig Follikulin enthaltenden Flüssigkeiten). *Zentrlbl. f. Gynäk.* 63: 506. 1939.

The well known Fluhmann technic for injecting blood serum into mice, after which the vaginal mucosae are sectioned and examined histologicalíy is subjected to test with simple aqueous solutions of estrogen, and the reaction is described in 4 stages: inactivity, proliferation, mucification and cornification, which occur in that order with increasing doses of estrogen. The number of stages is slightly different from the original author's description, but the results are an essential confirmation to Fluhmann's report. No data on application to any biological fluids appear. The method is a quantitative expression of increasing dose. The end point can be read with equal facility in 36 or 48 hours.—E. L. S.

Walther, H. W. E., and R. M. Willoughby. Hormonal treatment of benign prostatic hyperplasia. *J. Urology* 40: 135. 1938.

In 15 patients, treatment with injections of synthetic male hormone combined with the feeding of both the water-soluble and a liposoluble principle resulted in clinical improvement. It was necessary to continue treatment to maintain the improvement.—J. C. D.

Watson, B. P., P. E. Smith and R. Kurzrok. The relation of the pituitary gland to the menopause. *Am. J. Obst. & Gynec.* 36: 562. 1938.

Before laparotomies in 11 women, for repair of retroversion or removal of fibroids, the patients were injected with antex-leo, a gonadotropic extract from serum of pregnant mares. Ages ranged from 25 to 63, only 3 being menopause cases. Doses were 300 M.U. every 1 to 2 days from the end of a flow to the middle of the cycle. Operation followed soon afterward. When the ovaries reacted there were multiple follicle cysts, up to 2 cm. diameter, usually bilateral. Reaction by the ovary decreased with advancing age, and was absent in patients aged 43 to 67, in spite of large doses. Ova were not found in the follicle cysts, but some luteinization of theca and granulosa was seen at times, and in some uteri progestational changes were found. Two women showed stimulation of only one ovary. The conclusion is drawn that the menopause is due to loss of response by the ovary to gonadotropic hormones, and that one ovary may cease function before the other.—E. L. S.

Wattenwyl, H. v., The Nito reaction for diagnosis of early pregnancy (Die Reaktion nach Nito zur Frühdiagnose der Schwangerschaft). *Zentrlbl. f. Gynäk.* 63: 446. 1939.

Study of 30 cases demonstrated that this test for leukocytosis after intravenous injection of urine into a rabbit is not specific for pregnancy, and therefore not a useful substitute for accepted methods.—E. L. S.

Webster, B. The treatment of hypogonadism in the adolescent male. *J. Ped.* 13: 847. 1938.

The use of testosterone propionate is reported in 6 cases which included the following conditions: adiposogenital dystrophy, primary hypogonadism, bilateral cryptorchidism with previous operative procedures on hernia and testes and hypogonadism with severe constitutional disease. Striking improvement in growth of the penis, testes, scrotum and prostate was obtained. Secondary sex characters and voice change occurred as in normal puberty. The dose advised is 25 mg. of testosterone propionate 3 times a week.—M. B. G.

Weinstein, L., and J. Howard. The effect of estrogenic hormone on the H-ion concentration and the bacterial content of the human vagina, with special reference to the Döderlein bacillus. *Am. J. Obst. & Gynec.* 37: 698. 1939.

In 9 post-climacteric women vaginal secretions were studied before and after administration of

10,000-unit doses of theelin in oil. Vaginal acidity was much increased even in cases where the bacteria included no Döderlein organisms either before or after the therapy. The presence of the specific organisms is not necessary for production of acid, nor does it always result from the acidity. The production of acid is therefore due to enzymatic decomposition of glycogen, the latter being seceted by the mucosa under estrogenic stimulation.—E. L. S.

WESTERFELD, W. W., D. W. MACCORQUODALE, S. A. THAYER AND E. A. DOISY. The isolation of theelin from human placenta. *J. Biol. Chem.* 125: 195. 1938.

A 24-hour extraction with hot 95% alcohol was made of 422 kg. of human placentas which had been kept in a frozen state. The phospholipids and the cholesterol fractions were removed from the residue remaining after removal of the alcohol, and the estrogens removed by ether and sodium hydroxide. The ketonic and non-ketonic estrogens were separated by fractionation with Girard's reagent. The ketonic fraction yielded 12 mg. of pure crystalline theelin which accounted for 80% of the activity of the ketonic compounds present in the placentas.—J. M. L.

WESTERFELD, W. W., S. A. THAYER, D. W. MACCORQUODALE AND E. A. DOISY. The ketonic estrogen of sow ovaries. *J. Biol. Chem.* 126: 181. 1938.

The estrogenic material of sow's ovaries was extracted by 95% alcohol and after the removal of the alcohol the residue was freed from phospholipids and cholesterol, dissolved in ether and the phenolic portion shaken out with 0.5 N NaOH. The estrogens were separated into ketonic and non-ketonic fractions by Girard's reagent. It was found that the principal ketonic estrogen was theelin as evidenced by the similarity of reaction of these two substances in the following tests: *a*), inactivation by treatment with semicarbazide, *b*), partition ratios between 70% alcohol and benzene, *c*), ratios of weights of rat and mouse units, *d*), enhancement of activity by reduction of the ketone, *e*), partition ratios and rat unit-mouse unit ratios for the reduction product corresponding to dihydrotheelin. In a kg. of ovaries there were found 0.010 mg. of theelin equivalent to 20 R.U. and 0.014 mg. dihydrotheelin equivalent to 220 R.U.—J. M. L.

WILSON, R. B., L. M. RANDALL AND A. F. OSTERBERG. Studies on pregnandiol. *Am. J. Obst. & Gynec.* 37: 59. 1939.

The method of Venning was employed to determined pregnandiol content of urine in 5 cases with normal menstrual history, in 50 cases in which biopsy was performed on the endometrium of nongravid women who presented gynecologic complaints, in 10 cases of early gestation, in one case during the last month of gestation, and in 51 cases during the first week postpartum. The amounts of pregnandiol found in this group of patients with normal menstrual histories were essentially the same as those obtained by Venning in a similar group. Pregnandiol was found in significant amounts in the urine during the luteal phase of the menstrual cycle in a majority of the cases studied. Significant amounts of pregnandiol in the urine have been found during the stage of estrogenic activity when late proliferation existed in the endometrium. An abnormal microscopic appearance of the differentiated endometrium is nearly always associated with low values for urinary pregnandiol. The endometrial response to apparently normal hormonal stimulation may vary. Significant corpus luteum function, as evidenced by the amount of pregnandiol found in the urine, seems to be present on the fourth day postpartum in about 50% of the cases studied. The excretion of pregnandiol in normal or maximal amounts in the urine of a woman who has missed a period and who has had a normal menstrual history is apparently indicative of early gestation.—E. L. S.

WINTERTON, W. R., AND T. N. MACGREGOR. Clinical observations with stilbestrol (diethylstilbestrol). *Brit. M. J.* 1: 10. 1939.

The therapeutic value of the synthetic hormone stilbestrol was tested in a series consisting of 51 cases: 29 with post-menstrual disturbances, 12 with amenorrhea, 3 with dysmenorrhea, and 6 in whom it was desired to inhibit lactation. Stilbestrol was found to imitate the natural estrogens in its effects on the various forms of ovarian insufficiency, being as effective by oral administration as by the intramuscular route. It inhibited lactation in 5 mg. doses, without necessitating the usual procedures of bandaging the breasts, restriction of fluids, or purgation. Stilbestrol apparently prevents the liberation of the lactogenic hormone of the pituitary. Undesirable effects such as headache, nausea or vomiting appeared in some of the cases under treatment.—L. B. S.

WITSCHI, E., J. J. MAHONEY AND G. M. RILEY. Occurrence of prostatic lobes in the female rat. *Biol. Zentralbl.* 58: 455. 1938.

Prostatic lobes occurring in normal female rats are homologous to the most medio-ventral of the 5 segments which compose the ventral lobes of the male prostate. Two different strains of rats showed relative frequencies of 8.7 and 26.7% occurrence of prostatic lobes. Lobes are found much more often on

the right than on the left side. By the process of selective breeding a strain of rats can be developed with a high degree (77.3%) prostate incidence. The reaction of the female prostates to injected sex hormones corresponds in type and in degree to those of ventral lobes of the male prostates. Gross morphology and histology of the female prostate are of the castrate type. Females possessing such lobes are therefore not producers of increased amounts of androgenic hormones. It remains uncertain whether the formation of female prostates is determined by the appearance of high amounts of androgenic hormones during embryogenesis, or by non-hormonal conditions.—A. E. M.

Woodman, D. Output of prolan A in urine in certain extragenital conditions. *Brit. M. J.* 1: 666. 1938.

No increased excretion of prolan A occurred in the urine of patients having lesions of the breast or prostate as determined by the Aschheim-Zondek test. Increased excretion of prolan A was noted with chorionic epithelioma and teratoma testis, although negative results were found in cases of ovarian cyst, uterine fibroids and pituitary eosinophil adenoma.—H. J. D., Jr.

Zelson, C. Treatment of cryptorchidism with gonadotropic substance. *J. Ped.* 14: 453. 1939.

There is an aggregate in the literature of 468 cases of cryptorchidism with 682 undescended testicles which have been treated with gonadotropic substances. Response to treatment was obtained in 61% of the cases and in 63% of the testicles. The author reports his results in 26 boys ranging in age from 4½ to 12½ years. Of these, 18 showed unilateral and 8 bilateral involvement. Treatment consisted of the injection of APL preparation in doses varying from 100 to 500 R.U. 3 to 6 times a week; 1000 R.U. at one time were given to a small number of the patients. The total dosage varied between 1350 and 39,100 U. Complete descent occurred in 31% of the cases (8 cases). Of these, 5 were unilateral and 3 bilateral at the onset of treatment. Genital enlargement resulted in practically all of the cases. Hernia was present or developed in 5. Behavior disturbances were noted during the course of treatment in 7 instances. The author concludes that treatment can be expected to be successful within a period of three months and generally before 10,000 U are given. Massive doses are not necessary. Treatment should be instituted before 10 years of age.—M. B. G.

Zuck, T. T., and D. R. L. Duncan. The time of ovulation in the human female. *Am. J. Obst. & Gynec.* 38: 310. 1939.

The vaginal acidity was recorded daily for 3 consecutive cycles in 6 normal women, with the finding that there is a slight and brief shift toward alkalinity at the time of the ovulation, judging the latter by temperature shift, changes in vaginal smear and pains. In only 2 cycles was pregnandiol excretion determined as positive evidence of ovulation.—E. L. S.

Zunz, E., and J. LaBarre. Sex hormones and the control of glycemia (A propos de l'intervention des hormones sexuelles dans la régulation de la glycémie). *Compt. rend. Soc. de biol.* 129: 22. 1938.

Results of experiments on intact, hypophysectomized, thyroidectomized and vagotomized dogs and dogs with clamped suprarenal veins led to the conclusion that the hyperglycemia produced by estradiol, testosterone acetate, or progesterone was due indirectly to adrenaline secretion, this latter being elicited by thyroid stimulation, which in turn was evoked by release of anterior pituitary hormone induced by the sex hormones.—E. Cutuly.

HYPOPHYSIS

Schnitker, M. T., E. C. Cutler, O. T. Bailey and W. W. Vaughan. Chromophobe adenomas of pituitary; pathologic features and response to irradiation based on study of 81 verified cases. *Am. J. Roentgenol.* 40: 645. 1939.

This study of the chromophobe adenomas of the pituitary was made in order to evaluate the results of x-ray treatment. It has long been known that a certain percentage of the acidophile adenomas which give rise to acromegaly is benefited by radiation therapy. Similar benefit has been reported in patients with basophile adenomas. The report covers a careful clinical and pathological study of 81 verified cases. An attempt was made to correlate the histological findings with the history and with the results after surgery, after surgery and x-ray treatment, and with x-ray treatment alone. This investigation demonstrated that chromophobe adenoma of the pituitary may be satisfactorily treated with x-ray. The authors describe in detail the amount of therapy they use. From their experience they concluded that the more differentiated cell type of tumor, those where the cells are arranged in a normal sinusoidal form, and particularly when the tumor is avascular and solid, respond most satisfactorily to x-ray treatment. It is their opinion that unless there be urgent need for saving vision, patients with chromophobe adenomas should first be submitted to x-ray therapy.—From *J. Nerv. & Ment. Dis.*

Silberberg, M., and R. Silberberg. Effects of anterior pituitary implants and extracts on epiphyses and joints of immature female guinea pigs. *Arch. Path.* 26: 1208. 1938.

A total of 136 female guinea pigs weighing an average of 180 gm. were studied. Of these 14 received varying doses of acid extracts of anterior pituitary glands of cattle, ranging from 1 to 5 cc. over periods of 1 to 5 days. The animals were killed 1 to 2 days after the last injection and pieces of anterior lobes of

the pituitary were transplanted into 122 animals. The histologic characteristics of the various tissues in the epiphyses and in the joint were observed at different periods following the application of the transplants. The changes noted were swelling of the chondromucoid cartilage followed by, or associated with, atrophy and degeneration of the row of cells in the epiphyseal cartilage. These degenerations in the majority of instances were followed by extensive calcification. The hyperplasia and hypertrophy of the cells in the different layers of the epiphyseal cartilage, the chondrophyte and the cartilaginous covering of the joint took place. The authors concluded that the growth of the cartilage in all probability was a direct stimulation exerted by a substance which is present in the anterior lobe of the pituitary gland, rather than a regenerative process caused by degenerative changes.—L. B. S.

Silberberg, M., and R. Silberberg. Growth processes in cartilage and bone subsequent to gonadectomy and administration of anterior pituitary extract of cattle in immature male and female guinea pigs. *Am. J. Path.* 15: 55. 1939.

Histological study of certain epiphyseal lines and joints was made in 91 guinea pigs. Of these, 43 were males which were castrated when they weighed 135 to 160 gm. Some were killed 7 to 35 days later. Fifteen received daily intraperitoneal injections of 1 cc. of freshly prepared acid extract of anterior pituitary of cattle. These were killed after 7 to 28 days. Non-castrated animals also were injected and studied, while 8 served as normal controls. It was found that gonadectomy causes an increased proliferation of euhyaline cartilage. In males hyperplastic growth is accentuated, whereas in females there is a greater tendency to hypertrophy. The latter is especially evident within the joint, where arthropathic lesions are initiated more readily than in males. In males marked disturbances are observed in the zone of ossification together with a tendency to fibrosis of the bone marrow. A combination of the action of acid extract of anterior pituitary of cattle and of castration leads to a summation effect.—W. J. A.

Stadler, H. Relationship between periodic and episodic twilight and somnolent states and pituitary disturbances. *Monatschr. f. Psychiat. u. Neurol.* 98: 317. 1938.

The author has observed 6 male patients with acromegalic growth anomalies beginning at puberty and coinciding with the appearance of episodes of disturbed consciousness. In only 1 case evidence of a slight hydrocephalus was revealed by encephalography. Three patients suffered from somnolent phases only, which resembled normal sleep. They were reasonably well orientated. A 4th patient was deeply clouded as well as somnolent, and had amnesia for the affected period. In the 2 other cases phenomena suggestive of the epileptic ill-humor and twilight state were noted—wandering away, dreamy absorption, faulty orientation, automatism, somnambulism, amnesia, etc. After each episode these 2 patients were accustomed to sleep for 48 hours or longer, and then in contrast to the epileptic awake feeing refreshed and well. In 3 of the 6 cases, the affection appeared periodically every 4 weeks; in the other 3 the course was more or less chronic. According to the relatives, in almost all cases the patients manifested 1 to 2 days before the attacks a pallid, exhausted appearance, nervous, violent or entirely unusual conduct and irritability. There were no organic-neurological defects. On the other hand symptoms of vegetative stigmata and of general nervous hyperexcitability (accentuated reflexes, dermographismus, tremor, vagotonia, enlarged thyroid, etc.) were observed. The skin was hypersensitive to pain stimuli and titillation. One patient showed marked sexual frigidity. Hysteria and epilepsy could be excluded in all cases.—Abbreviated abstract, Science Library, Neuro Psychiatric Institute, Hartford Retreat.

Stehle, R. L., and S. M. Trister. Additional data concerning the chemistry of the pressor and oxytocic hormones of the pituitary gland. *J. Pharmacol. & Exper. Therap.* 65: 343. 1939.

Fractionation and purification by the method of Stehle and Fraser was applied to commercial posterior pituitary powder. The pressor preparation contained only arginine (yield 6.9%) of the three basic amino acids: arginine, histidine and lysine. Proline was present but hydroxyproline appeared to be absent. Isoleucine, cystine and tyrosine were identified by cysteine, methionine, trytophane and glycine appear to be absent. The constituent amino acids of the oxytocic hormone are same as those of the pressor hormone except that leucine instead of isoleucine is present.—E. L.

Stephens, D. J. Hypopituitarism. *Internat. Clin.* 1: 97. 1939.

Mild or incomplete grades of hypopituitarism occur more frequently than do such classical examples of pituitary failure as Simmonds' disease, pituitary dwarfism and infantilism. This paper deals essentially with details of 7 cases of acquired anterior pituitary deficiency in adults. An attempt is made to correlate the clinical findings with the important recent experimental observations relating to the various anterior pituitary hormonal effects. The predominant symptoms and signs in these patients included loss of appetite, weight and hair; arterial hypotension; genital atrophy; lowered basal metabolism, hypochloremia; hypoglycemia and increased carbohydrate tolerance. These abnormalities are interpreted as probable evidences of withdrawal of growth, gonadotropic, thyrotropic, adrenotropic and diabetogenic principles of the anterior hypophysis.—I. B.

Swann, H. G. Sodium chloride and diabetes insipidus. *Am. J. Physiol.* 126: 341. 1939.

In the permanent, but not the transient, phase of diabetes insipidus, the onset and continuance of the large fluid exchange are to a large extent dependent upon the ingestion of NaCl. If none is ingested, as in

fasting, or with diets low in NaCl, the diabetes insipidus is almost, but not entirely, prevented. It is suggested that the changes in water metabolism in diabetes insipidus are secondary to changes in NaCl metabolism.—Author's Summary.

TEAGUE, R. S., R. O. NOOJIN AND E. M. K. GEILING. The hypophysectomized frog (Rana pipiens) as a specific test object for melanophore hormone of the pituitary body. *J. Pharmacol. & Exper. Therap.* 65: 115. 1939.

Both normal and hypophysectomized frogs were used. Hypophysectomy was performed by a modification of Hogben's technic. The hypophysectomized frog is a specific test object for melanophore hormone of posterior pituitary extract if complete expansion of the melanophores is required as a positive test. Only pituitary extract, of the various drugs used, caused blackening of the hypophysectomized frog. Many other drugs, such as brucine, chloretone, metrazol and the barbitals produce marked darkening of the skin in normal frogs. Since these drugs do not act on the melanophores of hypophysectomized frogs, the authors believe their action is not peripheral.—E. L.

VAICHULIS, J. A. A simple method of removing the oxytocic principle from the posterior lobe of the beef pituitary glands. *J. Pharmacol. & Exper. Therap.* 66: 37. 1939.

The oxytocic principle is separated from the pressor principle by extracting with absolute alcohol the posterior lobe pituitary powder prepared from beef heads incubated for 20 to 24 hours at 37° C.—E. L.

WALLACE, E. W. Comparative assay of gonadotropic extracts of the pituitary upon the mouse and rat. *J. Pharmacol. & Exper. Therap.* 66: 38. 1939.

Under carefully controlled assay procedures, the mouse uterus was found to be 2 to 4 times as sensitive as the rat uterus. The uteri of both species were more responsive than the ovaries of either species. The fat ovary is more sensitive than the mouse ovary.—E. L.

WHITE, H. L., AND T. FINDLEY, JR. Responses of normal subjects and of patients with diabetes insipidus to water and salt ingestion. *J. Clin. Invest.* 18: 377. 1939.

Studies were made on 4 patients with diabetes insipidus and on some normals, in which the changes in blood chloride and protein concentrations following water, salt and posterior pituitary pressor fractions were alike. The differences were in urinary output, and can be explained as due to a reduced sensitivity of the kidney to environmental changes in diabetes insipidus. The authors find no justification for subdivision of diabetic cases into two types as described by Veil, or for any disturbance in other tissues than renal.—E. L. S.

WOLFF, E. Do either the hypophysis or the thyroid play a part in the development of experimental intersexuality in embryo chicks? (L'hypophyse et al thyroïde jouent-elles un rôle dans le déterminisme expérimental de l'intersexualité chez l'embryon de poulet?) *Compt. rend. Soc. de Biol.* 162: 1217. 1937.

By irradiation of very young chick embryos. it is possible to destroy the hypophysis or the hypophysis and the thyroid and still have development proceed. If such monsters are given doses of estradiol such as produce sex intergrades in non-irradiated chick embryos, they will become intergrades as is shown by the two cases reported. Neither thyroid nor hypophysis is, therefore, necessary for the action of this hormone.—J. C. D.

WOLFF, E., AND R. STOLL. The influence of the hypophysis on the embryonic development of chicks as shown by studies on experimental cyclocephalics (Le rôle de l'hypophyse dans le développment embryonnaire du poulet, d'après l'étude des cyclocéphales expérimentaux). *Compt. rend. Soc. de Biol.* 126: 1215. 1937.

By irradiation through a window in the shell, the heads of chick embryos can be damaged so as to produce cyclocephalic monsters without hypophysis. Such monsters continue to differentiate to the point of hatching. Their rate and degree of development does not differ materially from that of irradiated controls. Careful histological examination failed to show any interference with the growth of organs outside the injured area. For example, the adrenal developed normally and the thyroid showed characteristic colloid development.—J. C. D.

ZWECKER, I. T., Differences between castration cells and thyroidectomy cells of the pituitary of the rat in response to the administration of estrone and thyroid extract. *Am. J. Path.* 14: 773. 1938.

Rats were operated upon at 5 weeks of age and killed 5 weeks later. Forty-four were thyroidectomized and 34 were castrated. In each group some were injected with estrone, some were fed thyroid extract, and some were untreated. The lack of effect on thyroidectomy cells of estrone injected in a dosage greater than adequate to suppress castration cells, and the failure of castration cells to respond to thyroid extract in doses greater than required to suppress thyroidectomy cells indicate that thyroidectomy cells probably are structurally and functionally different from castration cells, whatever may be their derivation.—Author's Summary.

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